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ORIGINAL RESEARCH ARTICLE

Relationship of Tumor Necrosis Factor Super Family Member 4 (TNFSF4) Gene SNP Rs505922 with Atherosclerotic Cerebral Infarction in Tengzhou Region*

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ABSTRACT

Aim: To investigate the relationship between the incidence of atherosclerotic cerebral infarction and tumor necrosis factor super family member 4 (TNFSF4) on the upper point of the gene and the gene frequency SNP rs505922 polymorphism.

Methods: 185 patients with atherosclerotic cerebral infarction in local household registration hospital were seleated from in January 2012 to 12 January 2013, as the selected group, and the other selected 180 cases for routine checks in our hospital were selected as control group .The polymorphism and gene frequencies of SNP rs505922 on TNFSF4 were detected by polymerase chain reaction assa.

Results: rs505922 genotype and allele frequency analysis: C allele frequency in atherosclerotic cerebral infarction group was higher than that in the control group, the difference was statistically significant ($P < 0.05$). CC genotype is an independent risk of atherosclerotic cerebral infarction risk factors .

Conclusion: The upper point TNFSF4 gene SNP rs505922 CC genotype was closely related with local atherosclerotic cerebral infarction.

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At present, many scholars agree that genetic and environmental factors play an important role in the development process in cerebral infarction, but its pathogenesis still remains unclear.[1] Cerebral infarction can be divided into atherosclerotic cerebral infarction and lacunar cerebral infarction on the basis of different cause. [2] Atherosclerosis cerebral infarction refers to cerebral infarction that appear on the basis of cerebral atherosclerosis vascular lesions such as vascular stenosis, occlusion and thrombosis that are caused by cerebral infarction in corresponding blood vessels. Lacunar cerebral infarction refers to the small deep cerebral hemisphere or brain stem through artery (100-200 microns in diameter), On the basis of long-term high blood pressure, blood vessel walls lesions occur, causing lumen out-of-the-way and forming small brain infarcts. According to Gu Shuiming's[3]study found, atherosclerosis is the most common cause for cerebral infarction, tumor necrosis factor super family member of 4 (TNFSF4) gene's polymorphism and the development of atherosclerosis are closely related, rs505922 is the important regulating site on gene TNFSF4, but at the moment about the relationship between rs505922 gene's polymorphism and frequency and the atherosclerotic cerebral infarction incidence is still missing report at home and abroad. For the above reason, this article will detect the rs505922 gene among regional atherosclerosis cerebral infarction, and compared it with normal healthy people and dedicated to provide a new research idea for screening high-risk patients with cerebral infarction.

Materials and Methods

Equipments and Materials PCR Automatic Amplifier (GeneAmp PCR System 3700, American PERKIN ELMER), DNA Sequencing machine (Prism 3700 DNA Analyzer 377, America, ABI).

The research objects and grouping Select 185 cases of local household registration of ischemic cerebral apoplexy patients treated by Central people's hospital neurology Department in Tengzhou, Shangdong province between January 2012 and December 2012 as research objects, among which 98 were male, 87 cases were female , aged from 38 to 75 years old, average age (63.52± 4.36) and 88 cases were of atherosclerosis cerebral infarction, and 97 cases were of lacunar cerebral infarction.The other selected 180 cases were of Tengzhou, Shandong province's household health checks, 92 cases were male and 88cases were female, aged 22 to 76 years old, average age(61.22± 9.41).
The standard set for grouping. Inclusion criteria (1)All cases were with typical clinical manifestations, by craniocerebral CT and/or MRI diagnosis, all selected objects are in line with the 2010 session of the national ninth cerebrovascular revision of the diagnostic criteria.[4] (2)Control group by clinical examination to rule out disease of heart head blood-vessel, liver function, renal function were normal healthy check-up.

Exclusion criteria. Incomplete liver and kidney function, severe heart failure, increased intracranial pressure, pneumothorax, shock, thoracic trauma, cardiac, arteritis, drugs, tumor, brain aneurysms, vascular malformations caused by cerebral infarction patients.

Data collection. To collect two groups of subjects' gender, age, BMI, history of coronary heart disease history, history of hypertension, diabetes mellitus, history of smoking, drinking, FPG, TC, LCL - C, TG, HCL - C and other

Table 1 clinical data of ischemic stroke group and control group

General Situation	Ischemic stroke group (n=185)	Control group (n=180)	χ2/t	P value
Gender(Male/Female)	98/87	98/82	0.712	0.396
Average age(Year)	63.52±4.36	62.89±3.45	0.326	0.712
Coronary heart disease	22(11.89)	2(1.08)	8.263	0.000
Diabetes	26(14.05)	2(1.08)	7.962	0.000
Smoking history	98(52.97)	32(17.30)	9.123	0.000
Drinking history	102(55.14)	27(14.59)	10.145	0.000
BMI(kg/m2)	27.26±2.18	24.98±2.36	4.265	0.007
FPG(mmol/L)	6.32±1.85	5.23±0.98	4.985	0.006
TC(mmol/L)	4.98±0.87	4.21±0.96	5.262	0.003
TG(mmol/L)	2.65±0.45	1.02±0.37	5.142	0.004
LCL-C(mmol/L)	2.98±0.58	2.02±0.32	5.986	0.000
HCL-C(mmol/L)	1.07±0.75	1.39±0.41	5.023	0.008

indicators.

There was no significant difference on average age and gender composition between Ischemic stroke group and the control group, however, Ischemic stroke group have more proportion of patients with high blood pressure, diabetes, coronary heart disease, triglyceride, total cholesterol and other risky factors than the control group (P<0.05) (Table1)。

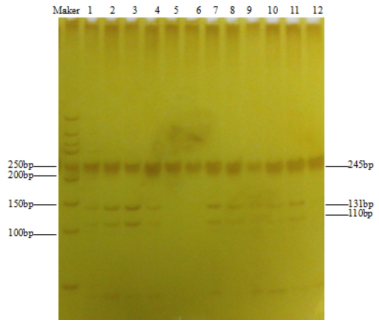
TNFSF4 Genetic type detection[5]

Morning fasting venous 2 ml blood in patients, put in EDTA anticoagulant tube in - 20 ℃ refrigerator save for later use.Use blood genomic DNA extraction kit to extract DNA. Polymerase Chain Reaction (PCR)primer application Oligo and Premier software to design primers, by Sangon Biological Engineering (Shanghai) co., LTD, Upstream:5′ - GAGC CAAGACTTGTCCCCGG - 3′, Downstream:5′ - GAGCCAAGACTTGTCCCCGA - 3′ ,conducting polymerase chain reaction。Amplification reaction system (25ul):2× Taq Master Mix 12.5ul, 1ul primers for upstream and downstream respectively, DNA model3ul, deionized water7.5ul。Reaction conditions: 94 ℃ degeneration 3 min, 94 ℃ modified 30 s, 30 s, 60 ℃ annealing extension 72 ℃ for 1 min;After 30 cycles, 72 ℃ for 10 min. The PCR product detection using 2% agarose gel, 120V electrophoresis for 30-40min, Imager ethidium bromide color, gel electrophoresis banding photographic observation (Fig1), After amplification product size is of 245 bp.

PCR products enzyme reaction system (20ul):PCR products 8ul, NIa Ⅲ 2 ul, restriction enzyme BtsI ul and 9.5 ul deionized water. 55 ℃ water bath enzyme 1 hour after polyacrylamide gel electrophoresis, 220V electrophoresis (220-220 min, fixed, dyeing, color, gel image analysis system analysis as genotypes *(Fig1)*。

Fig1 The polyacrylamide gel electrophoresis map of TNFSF4 gene extron Rs505922 digested by enzyme NIaⅢ.

Notes: The M lane is 50-450 bp DNA ladder; 1-6 lanes are the experimental group of agarose gel electrophoresis; 7-12 lanes are the control group of agarose gel electrophoresis



Statisti cal method of credit

Using SPSS1 9.0 software analysis the result of the experiment, measure ment data using mean standard deviation (+ / - s) to mani fest,com parison be tween measured data set using t test,using χ 2 test between count data sets,single factor analysis using Pearson, multi ple factors using Logistic analysis, P < 0.05 has statistical significance.

Results

1. Ischemic stroke subtype group compared with control group genotypes and alleles

In 185 cases of acute cerebral infarction group, there were 88 cases of subtypes atherosclerosis cerebral type (CT) infarction and 97 cases of subtypes lacunar cerebral infarction (LI). Atherosclerosis in patients with cerebral infarction group CC, CT proportion and, C allele frequency is higher than the control group, the difference was statistically significant (P<0.05), The lacunar cerebral infarction group however, when compared with control group, CC, CT proportion and, C allele frequency has no statistic significance(P>0.05), Fig.3.

dent risky factors, among which the highest risk being the CC genotype (OR:4.986, 95% CI:1.232~7.025), Table3.

Table 2 Ischemic stroke subtype group rs505922 genotype and gene frequency analysis

category		Genotype [case (%)]			Allele frequency		genetype		Allele frequency comparison				
Group	(n)	CC	CT	TT	C	T	χ2	P	χ2	P	OR	(%)	CI
(CT)	88	33 (37.50)	46(52.27)	9(10.23)		0.892	9.123	0.000	5.693	0.013	1.185	95%	0.936 ~ 3.245
(LI)	97	5(5.15)	12(12.37)	80(82.47)	0.182	0.824	1.695	0.102	1.245	0.321	1.245	95%	1.145 ~ 3.696
control group	180	12 (6.67)	20(11.11)	148 (82.22)	0.185	0.815							

Table 3 Logistic analysis of atherosclerotic cerebral infarction risk factors

Risk factors	Regression coefficient	OR(95%CI)	Pvalue
CC	1.325	4.986(1.232–7.025)	0.000
CT	1.428	3.425(1.563–4.252)	0.058
TT	1.396	1.258(0.963–4.158)	0.063
High blood pressure	1.262	4.125(1.136–5.912)	0.012
Coronary heart disease	1.189	3.785(1.063–5.126)	0.015
Diabetes	1.525	3.458(1.063–5.023)	0.019
Smoking history	1.325	1.345(1.152–6.023)	0.068
Drinking history	1.316	1.258(0.856–5.102)	0.071
FPG	1.242	1.312(1.023–5.639)	0.075
TC	1.278	1.212(1.102–5.412)	0.079
TG	1.112	1.362(1.245–6.325)	0.085
LCL–C	1.028	1.247(1.045–6.102)	0.091
HCL–C	1.013	1.158(0.912–6.352)	0.093

Discussion

Cerebral infarction is a kind of disease that caused by many factor like genetic factors and environmental factors, mainly for the elderly, with high mortality and morbidity[6]。

A large number of epidemiology, etiology research suggests genetic factors play a vital role in the process of the occurrence of cerebral infarction.The recent experiment suggests that human TNFSF4 gene mutation are inextricably linked with the occurrence and development of atherosclerosis. [7-9] Gene TNFSF4is also called tumor necrosis fair super family member 4，has length about 23．60Kb，locates in l q 25，has 3exons，Coding OX40L protein (also called OX40 Ligand, CDl34L, GP34, TXGPI).OX40L protein mainly expressed in dendritic cells, B lymphocyte and vascular endothelial cells，CD40 signal can promote its expression. [10] OX40L can combined with Ox40 receptors on T lymphocyte, thus to stimulate signals，and eventually lead to the proliferation and differentiation of T lymphocyte ，and participate in memory CD4 + T cell's differentiation and mature, mediate T cell to inflammatory infiltrates.[11、12]

OX40L is TNFSF4 gene's encoding protein, OX40L can code B lymphocytes, dendritic cells, endothelial cells, macrophages, and some tissue cells.Sun Shuntao et al.[13] study indicates that OX40L can participate in the process of atherosclerosis by promoting T lymphocyte function.Li Junnan et al.[14]By in vitro experimental mice a high-fat diet，the result shows that mice with TNFSF4 genotype are more likely to develop atherosclerosis disease when compared with mice with non TNFSF4 genotype，thus infer gene TNFSF4 potentially exist in the control area of single nucleotide polymorphism can affect the potential gene expression, thus affect the process of atherosclerosis. At present, a lot of in vitro animal experiments have confirmed that TNFSF4 is currently affect the susceptibility genes of atherosclerosis. Su Rui et al.[15] To look at the myocardial infarction and normal people, the result shows that gene TNFSF4 SNP rs505922 patients are more likely to happen with myocardial infarction. Wang Kechun et al [16] study found that patients with type 2 diabetes，Gene TNFSF4 SNP rs505922 carriers of atherosclerosis and plaque formation is significantly higher than the proportion of the SNP rs505922 carriers.

Rs505922 locates in the third intron area of gene TNFSF4's extra no.9 middle reaches area. This study found，Gene TNFSF4 SNP rs505922 polymorphism may participate in the onset process of cerebral infarction patients in Tengzhou, Shandong province，and plays vital role in this process. In this research, both control group patients and atherosclerotic cerebral infarction group have three genotypes of CC, CT and TT, two groups of rs505922 gene polymorphism go with Hardy - Weinberg balance check, thus to prompt that the samples are from the same population，gene frequency can represent the group distribution，the atherosclerotic cerebral infarction group C's allele frequency is higher than the control group，this difference has statistic significance （$P<0.05$），prompting that rs505922 may be closely related to the regional atherosclerosis cerebral infarction incidence，thus can deduce，base substitution can affect gene TNFSF4's promoter and the expression level of mRNA，and can also enhance the expression

level of OX40L in some way so as to activate OX40L signal path, enhance the expression level of T cells in the body, stimulate the body to massively release a variety of cytokines, mediating body's various inflammatory response and promote the formation of atherosclerosis.

By analysing Logistic multifactors, the CC genotype is an independent risky factor for atherosclerotic cerebral infarction. Recent studies pointed out that coronary heart disease (CHD) soluble OX40L were significantly higher than that of healthy people, thus to infer that rs505922 can, affect the plasma level of soluble OX40L, promote atherosclerosis and increase the risk of cerebral infarction by regulating gene function. This study found that, the CC genotype patients are more prone to have atherosclerosis cerebral infarction, we should strengthen the clinical prevention and treatment of cerebral infarction patients, reduce the incidence of atherosclerosis cerebral infarction and the morbidity.From what has been discussed above, SNPS rs505922 CC genotype locates on Gene TNFSF4 is closely related to the atherosclerosis cerebral infarction in Tengzhou, Shandong province, SNP rs505922 C allele can be used as independent risky factors of atherosclerosis cerebral infarction in Tengzhou, Shandong province.In view of the limited sample size of this study, and the ethnic and regional differences in the correlation of gene and disease, later researchers need to further expand the sample size in different regions and population to reveal the pathogenesis of ischemic cerebral apoplexy, to further in-depth study TNFSF4 exon genetic region rs505922 polymorphic loci's role in ischemic stroke.

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