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RESEARCH ARTICLE

APOE GENOTYPES AND CEREBRAL MICROBLEEDS ON MRI IN HAN CHINESE POPULATION

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Abstract

The relation between APOE genotypes with cerebral microbleeds (CMBs) was investigated on the basis of the location of CMBs in 569 patients with ischemic stroke. With respect to the ε2 or ε4 allele carrier, the adjusted odds ratio was 1.87 (1.06 to 3.28) for lobar CMBs but 1.21 (0.82 to 1.87) for nonlobar CMBs. These analyses revealed that the ε2 allele may contribute to the genesis of lobar CMBs to a greater extent than the ε4 allele, instead of nonlobar CMBs. These results suggest that the pathogenesis of CMBs may differ depending on not only the frequency and distribution of CMBs but also their association with the APOE genotypes.

Introduction:-

Cerebral microbleeds (CMB), as one of the multiple manifestations of cerebral small vessel diseases, which were first reported in the late 90s of last century and played an important role as an imaging biomarker notably in vascular and neurodegenerative diseases. CMB, as a foci of past micro hemorrhage containing deposits of hemosiderins, appear as small and round hypointense foci susceptibility weighted imaging (SWI) MRI [1].Presence of APOE ε2 or ε4 alleles is associated with cerebral amyloid angiopathy–related hemorrhage [2] and has recently been considered as the risk for lobar hemorrhage [3].Cerebral microbleeds are the presence of a group of pathological processes affecting the small arteries, arterioles, capillaries, and venules of the brain.

Investigating the relationship between APOE genotypes and CMBs can contribute to clarify the underlying mechanism of cognitive impairment. Presence of an APOE ε4 allele is the only genetic factor involved in the processing which could be increasing the risk of CMB development. High-quality meta-analytic report (24 studies, n=8546) recommended that there was no association between APOE ε4 allele and WMH [4]. And another meta-analysis (11 studies, n=8917) suggested that APOE ε4 allele carrier significantly associated with increased WMH and CMBs, and APOE ε2 alleles carrier was significantly related with increasing WMH load [5][6]. This suggests that racial differences can not only induce differences in CMBs frequency and distribution but also differences in their association with APOE genotypes [7,8]. Recent study included 454 individuals composed by 176 subjects with cerebral microbleeds and 278 subjects without cerebral microbleeds in a non-Hispanic/Latino white population was performed. ApoE ε4 was confirmed independently associated with the presence and progression of cerebral microbleeds [9]. Recently, in Japanese participants, APOE ε4 allele was a significant risk factor for lobar CMBs but not for deep/infratentorial CMBs [10]. This implies that APOE genotypes such as ε2 or ε4 alleles may contribute to the occurrence of lobar CMBs. While the relation between APOE genotypes polymorphism and CMBs on susceptibility weighted imaging MRI in Han Chinese population is still unknown.

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Methods:-

Study Population:

A consecutive series of 569 patients who were admitted to the Department of Neurology, had reveived brain MRI examination, and had consented to participate in this study, were recruited prospectively. The neurologic abnormalities were stroke or TIA (70%), followed by vertigo, limb weakness, speaking clumsy, dysarthria, visual impairmen, dementia, etc. This study was conducted with the approval of the Qingdao University ethics committee. Written informed consent was obtained from all participants. CMBs were observed on SWI-MRI, as homogeneous round signal loss lesions with a diameter up to 5 mm. The location of CMBs is divided into lobes and non-leaves (basal ganglia, thalamus, brainstem and cerebellum). If early confluent or confluent on fluid attenuated inversion recovery images, it is judged that there is leukoaraiosis. MRI was assessed by blinded clinical and genetic information. Clinical characteristics were defined and collected as follows: age, gender, smoking (current smokers or smokers who quit smoking within 5 years), moderate alcohol consumption (drinking >3 days/week and > 1 cup/day), hypertension (treated or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg), diabetes (treatment or fasting blood glucose > 140 mg/dL), hyperlipidemia (treatment or total cholesterol> 240 mg/dL or low-density lipoprotein cholesterol > 160 mg/dL), ischemic heart disease, history of stroke and currently used antithrombotic drugs. Antithrombotic medication included both antiplatelets (aspirin, clopidogrel, ticlopidine, and cilostazol) and anticoagulants (warfarin and direct oral anticoagulants). APOE polymorphism was determined by genotyping two single nucleotide polymorphisms (rs429358 and rs7412) using a multiplex PCR-based Invader assay provided by Nanjing Dongji Biotechnology Co., Ltd. The APOE genotype was determined by the PCR-restriction enzyme method and was classified as carrying the APOE ε2 or ε4 allele vs without carrying the alleles.In brief, DNA fragments were amplified separately, using the following primer pairs: 5'-TGTCCAAGGAGCTGCAGG -3',5'- CTGCCCATCTCCTCCATCC -3' for APOE rs429358r(393bp), and 5'-ATGCCGATGACCTGCAGAA', 5'- CTGCCCATCTCCTCCATCC -3' for APOE rs7412(219bp). Those who carried at least 1 copy of the e4 allele were categorized as APOE ε4 carriers. The participants with at least 1 copy of the ε2 allele were defined as APOE ε2 carriers[P].Primers and reagents are from dongji biological co. LTD.The association between CMBs with each of the demographic, clinical, or radiologic variables was analyzed, and variables with $p<0.2$ were chosen for adjustments. As a dependent variable, crude and adjusted odds ratios (ORs) of APOE genotype such as ε2 or ε4 allele possession and 95% CIs were estimated by logistic regression analyses using the presence of CMBs in any location. Similar analyses were repeated using the presence of lobar CMBs and nonlobar CMBs as dependent variables. $p<0.05$ was considered significant.

Results:-

A total of 99 subjects (17.4%) had CMBs: 12 (2.1%) had only lobar CMBs, 32 (5.6%) only nonlobar CMBs and 57 (10.0%) had CMBs in both locations. Comparisons of the characteristics between subjects with and without CMBs are presented in Table 1, and the frequencies of the APOE genotype based on CMB location are presented in Table 2. The proportion of subjects carrying the APOE ε2 or ε4 allele might differ according to the location of the CMBs. The APOE ε2 or ε4 allele was present in 7 of 12 subjects that had lobar CMBs (58.3%), 9 of 32 subjects that had nonlobar CMBs (28.1%), and 22 of 57 subjects that had CMBs in both locations (38.6%). The present study revealed that the prevalence of CMBs was 17.4% in the Chinese patients with ischemic stroke.

Table 1:- Comparisons of demographic, clinical, and radiologic characteristics between subjects with and without CMBs.

	CMBs in any location		
	CMBs(n=99)	nCMBs (n=470)	P*
Age,y	66.6±10.5	67.3±13.2	
Male	51(51.5%)	221(47.0%)	0.416
Smoker	39(39.4%)	154(36.0%)	0.267
Heavy alcoholic	11(11.1%)	44(9.3%)	0.782
Hypertention	73(73.7%)	212(45.1%)	0.000
Diabetes mellitus	30(30.3%)	162(34.4%)	0.486
Hyperlipidemia	35(35.3%)	128(27.2%)	0.181
Ischemic heart disease	7(7.0%)	39(8.3%)	0.847
History of stroke			0.032
None	30(30.3%)	210(44.6%)	
Ischemic only	58 (58.6%)	192(40.9%)	

Hemorrhagic	11(20.2%)	68(14.5%)	
Leukoaraiosis	70(70.7%)	150(31.9%)	<0.0001
Current use of antithrombotics	55(55.5%)	200(42.5%)	0.682

The values were calculated be the Mann-Whitney U test, Peason test, or Mantel-Hanszel test for lineart disease
CMBs=cerebral micobleeds

Table 2 shows the OR carrying the APOE ε2 or ε4 alleles, which were estimated for the presence of CMBs in any location, in lobar locations, and in nonlobar locations. Posthoc analysis was performed to isolate the effects of the ε2 and ε4 alleles.

Table 2:- APOE genotypes according to CMBs location.

	CMBs location		
	Any	Lobar	Nonlobar
ε2 or ε4 allele			
Frequency*			
CBMs(+)	38/99(38.3%)	29/69(42.0%)	31/89(34.8%)
CBMs(-)	140/470(29.8%)	176/602(29.2%)	174/578(30.1%)
[a]OR	1.42(0.94-2.13)	1.54(1.04-2.68)	1.02(0.98-1.06)
[b]Adjusted OR	1.60(0.92-2.77)	1.87(1.06-3.28)	1.24(0.82-1.87)
ε2 allele			
Frequency*			
CBMs(+)	20/99(20.2%)	16/69(23.2%)	16/89(17.9%)
CBMs(-)	64/470(13.6%)	78/602(13.0%)	78/578(13.5%)
[a]OR	1.50(0.93-2.40)	1.67(1.08-2.57)	1.03(0.98-1.08)
[b]Adjusted OR	1.88(0.90-3.93)	2.41(1.15-5.06)	1.40(0.78-2.51)
ε4 allele			
Frequency*			
CBMs(+)	19/99(19.2%)	15/69(21.7%)	15/89(16.8%)
CBMs(-)	80/470(17.0%)	105/602(17.4%)	103/578(17.8%)
[a]OR	1.18(0.63-2.20)	1.33(0.70-2.50)	1.01(0.92-1.10)
[b]Adjusted OR	1.29(0.49-3.38)	1.51(0.60-3.77)	1.05(0.68-1.65)

[*]Values incicates the number of patients with the specific APOE type over the number of patient with or without cerebral cerebral micobleeds (CMBs)

[a]Values are odds ratio(ORs) (95%CI)
[b]Values are odds ratio(ORs) (95%CI),adjusted for age,sex,hypertention,diabetes mellitus,hyperlipidemia,ischemic heart disease,history of stroke, current use of antithrombotics.

Discussion:-

The Framingham study reported that there was no correlation between any of the APOE alleles and the presence of CMBs in any location as well as in lobar locations. Another report from Korea University suggested that APOE ε2 or ε4 alleles carrier might contribute to the occurrence of lobar CMBs.The APOE genotype was associated with lobar CMBs after adjustments, although it was not associated with nonlobar CMBs. This suggests that the pathogenesis of CMBs may differ depending on their location [11].

In the present study, the APOE genotype was associated with lobar CMBs after adjustments, although it was not associated with nonlobar CMBs. The positive association between the APOE genotype and lobar CMBs was supported by the data that the percentage of genotypes carrying the ε2 or ε4 allele was the highest in patients with only lobar CMBs (58.3%) compared with patients without CMBs (29.2%), those with nonlobar CMBs (28.1%), and those with CMBs in both locations (38.6%). Discrepancies between our study and the previous studies may be due to several reasons. First, this is probably due to stroke patients examples in our study. Second, there might be racial differences can not only induce differences in the frequency and distribution of CMBs as well as APOE genotypes. Based on the closely relation between lobar hemorrhage and the ε2 or ε4 allele carrier reported in a previous study[3]. We also defined the APOE genotype and analyzed them together in the same way as discribed in the the previous studies [7,11]. As post-hoc analyses, the effects of ε2 and ε4 alleles were separately examined. These

analyses revealed that the ε2 allele may contribute to the genesis of lobar CMBs to a greater extent than the ε4 allele. Previous findings suggested APOE ε4 allele was associated with increased Aβ deposition, which may lead to the formation and progression of WMH, especially in frontal lobe [12]. There are significant relationships between CMBs with Aβ load [13].This can not explain why the ε2 allele, not ε4 allele is closely related to lobar CMBs which has been obtained in the present study. It is speculated that there may be some other mechanism related to APOE ε2, which is involved in the occurrence of CMBs. These results were in a harmony with previous study in the Korea population [11],which were not completely same to that in Framingham study[7]. We think that the issue of racial difference might contribute to these different results.The Framing-ham cohort was mostly Caucasian, and the Korea University cohort was ethnically Korean,whereas our subjects were ethnically Han Chinese. Racial difference could induce not only a difference in the frequency and distribution of CMBs but also a difference in their association with the APOE genotypes.

There are several limitations in this research. This is a cross-sectional study based on a hospital admitted to the hospital. There was undeniable heterogeneity in the composition of the subjects because of the single iscemic stroke population. Whether the acute pathological process of cerebral infarction may affect the occurrence and quantity of CMBs, it still remains unclear. The results should be interpreted carefully and further research is needed.

Compliance with Ethical Standards:

Funding:
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Conflict of Interest:
All the authors declare that they have no conflict of interest.

Ethical approval:
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study has been approved by the Ethics Committee of Qingdao University.

Informed consent:
Informed consent was obtained from all individual participants included in the study.

Data Availability Statement:
The original data data used to support the findings of this study are currently under embargo. Requests for data, [6/12 months] after publication of this article, will be considered by the corresponding author.

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