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Original Article

ALDH2*2 polymorphism is associated with an increased risk of extra cranial vascular stenosis and poor collateral vessels in ischemic stroke in Han Chinese

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Abstract: *Background:* Human aldehyde dehydrogenase 2 (ALDH2) is the major oxidative enzyme in alcohol metabolism. The ALDH2*2 polymorphism modifies the activity of this enzyme. This study assessed the association of *ALDH2*2* with ischemic stroke and cerebral vascular stenosis. *Methods*: A total of 394 patients with acute ischemic stroke and 406 healthy controls were recruited for *ALDH2* genotyping using Polymerase Chain Reaction (PCR) and DNA sequencing. Cerebrovascular stenosis was evaluated using digital subtraction angiography (DSA). *Results: ALDH2* genotype and allele frequency did not differ between stroke patients and healthy controls (*P*=0.71 and 0.69, respectively). Moreover, there was a significantly lower frequency of the *ALDH2* AA genotype in patients with abnormal intra cranial (IC) arteries (*P*=0.03, *OR 0.18*, 95% CI 0.04-0.81), whereas the AA genotype frequency was significantly higher in patients with abnormal extra cranial (EC) arteries (*P*=0.03, *OR 6.05*, 95% CI=1.16-31.53). The rate of good collateral vessels was significantly lower in the mutation group (GA+AA) (*P*=0.001, *OR 3.00*, 95% CI=1.54-5.82) and the allele frequency was significantly different between these two subgroups (*P*=0.02, *OR 1.86*, 95% CI=1.09-3.16). *Conclusions:* The data from the current study suggests that *ALDH2*2* is a protective factor for IC arteries and a destructive factor for EC arteries in patients with ischemic stroke in this Han Chinese population. The rate of good collateral vessels was significantly lower in the mutation group (GA+AA and A allele).

Keywords: Aldehyde dehydrogenase 2, digital subtraction angiography, polymorphism, brain ischemic, cerebral vascular stenosis, collateral vessels

Introduction

The term “stroke” refers to a cerebrovascular accident that occurs due to an interruption of blood supply to the brain which leads to dysfunction or death of brain cells [1]. To date, stroke is the second leading cause of global human mortality [2], the incidence of which is increasing considerably in the developed world due to an ageing population [1]. In China, the most populous developing country, stroke accounts for approximately 20% of all deaths [3, 4]. Etiologically, stroke can be divided into ischemic and hemorrhagic stroke and the former is the predominant form of stroke in China [5]. Stroke caused by intracerebral hemorrhage usually occurs due to bursting of small arteries or arterioles, which is commonly induced by hypertension or intracranial vascular malformation [6]. In contrast, ischemic stroke is caused by blockage of blood flow into the brain, which can lead to dysfunction and death of brain tissue. There are four different causes that lead to blood flow blockage into the brain; thrombosis, embolism, systemic hypoperfusion and venous thrombosis [1]. Atherosclerosis of extracranial or intracranial arteries in the brain usually results in thrombosis or hemorrhage. Previous studies showed that the distribution of carotid atherosclerosis exhibits racial and/or geographical trending, which has been confirmed in autopsy and angiographic studies [7]. For example, in Caucasians, extracranial atherosclerosis accounts for the majority of stroke cases. However, intracranial atherosclerosis is the most common cause of stroke in Asians

and blacks [7-9]. Other studies have indicated that genetic risk factors also play a role in the development of atherosclerosis [10, 11]. Thus, further studies investigating genetic variants could facilitate our understanding of the interaction between genetic factors and the environment and the associated potential for stroke prevention.

Human aldehyde dehydrogenase 2 (ALDH2) is a 517-amino acid enzyme encoded by a gene localized at chromosome 12q24 [12]. ALDH2 is expressed ubiquitously in all tissues but is most abundant in the liver and in organs that require high mitochondrial oxidative phosphorylation, such as the heart and brain [13, 14]. ALDH2*2 expression is found in about 560 million East Asians and approximately 8% of the rest of the global population [15]. A previous study demonstrated that substitution of glutamic acid 504 to lysine could result in a polymorphism of *ALDH2* [12]. Due to the fact that there are two ALDH2 alleles (*1 and *2) in human cells, the potential substitution combinations are *1/*1 (GG, typical homozygote), *1/*2 (GA, heterozygote), and *2/*2 (AA, atypical homozygote) [16]. The protein encoded by mutant *ALDH2* *1/*2 and *2/*2 exhibits significantly reduced catalytic activity in relation to acetaldehyde metabolism [17]. The ALDH2*2 variant has been implicated in various human diseases, including neurodegenerative diseases [18-21]. Previous studies also showed that *ALDH2* mutation was related to the severity of carotid and coronary atherosclerosis [22, 23]. In our previous study, we reported that *ALDH2*2* could be a protective factor in the prevention of severe intracranial vascular stenosis following magnetic resonance angiography (MRA) in ischemic stroke patients [24]. However, research has not yet demonstrated an association between ALDH2*2 and ischemic infarction or an association between the occurrence of vascular lesions and the degree of severity of clinical manifestations. Thus, in this study, we assessed *ALDH2* polymorphism for association with acute ischemic stroke. We then explored the correlation between *ALDH2* mutation and vascular stenosis location and severity. We also investigated the relationship between the occurrence of ALDH2*2 and cerebral collateral circulation. This study could provide the basis for the future use of *ALDH2* polymorphism in stroke prediction and a potential strategy in the treatment of ischemic stroke.

Materials and methods

Study population

Between January 2014 and September 2015, we recruited 394 acute ischemic stroke patients. The patients were recruited from Yuhuangding Hospital (Shandong, China) with confirmed stroke occurrence facilitated using magnetic resonance imaging (MRI). The inclusion criteria were: 1). All subjects were diagnosed with acute ischemic stroke according to the World Health Organization's stratified criteria for stroke; 2). They were diagnosed as ischemic stroke patients using brain MRI; 3). They were ≥18 years of age; 4). They were first time stroke patients; and 5). The patients or their guardians provided informed consent before participation in this study. The exclusion criteria were as follows: 1). Patients had cardioembolism; 2). Patients had an intracranial hemorrhage, transient ischemic attack, cerebral vascular malformation, or diseases other than ischemic stroke to account for neurological deficits; 3). Patients had received anticoagulation therapy with a bleeding diathesis, had a history of illicit drug use, had concomitant serious medical illness, such as malignancy, uremia, liver cirrhosis, sepsis, meningoencephalitis, autoimmune disorders, and vasculitides; 4). Patients were defined as "heavy drinkers" consuming 210 g/week or more [25]; and 5). They refused the digital subtraction angiography examination. The controls were recruited from the same hospital during the same time-period. The control group consisted of 406 gender- and ethnicity-matched healthy volunteers living in the same area with no history of stroke. And the ALDH2 genotypes of these people were in Hardy-Weinberg equilibrium. This study was approved by the Ethics Committee of Yuhuangding Hospital.

Clinical data collection and evaluation

The medical history of patients and controls was collected and included information pertaining to age, gender, history of hypertension (defined as receiving medication for hypertension or blood pressure >140/90 mmHg with repeated measurements), history of diabetes mellitus (defined as receiving medication for diabetes mellitus, fasting blood sugar ≥126 mg/dL or post-prandial 2 hours ≥200 mg/dL after two separate measurements), and family

Table 1. Characteristics of studied populations

	Patients	Controls	*p* value*
N	394	406	
Age, yr. (mean ± SD)	60.21 ± 10.73	60.99 ± 8.02	0.41
Male, n (%)	284 (72.1)	280 (69.0)	0.49
Hypertension, n (%)	262 (66.5)	232 (57.1)	0.054
Diabetes mellitus, n (%)	150 (38.1)	114 (28.1)	0.034
Family history of cerebrovascular disease, n (%)	118 (29.9)	84 (20.7)	0.033
Cholesterol, mM (mean ± SD)	4.97 ± 0.99	4.75 ± 1.04	0.033
Triglyceride, mM (mean ± SD)	1.56 ± 0.84	1.53 ± 0.88	0.71
Uric acid, mM (mean ± SD)	293.98 ± 84.05	306.92 ± 86.74	0.13
Homocysteine, μM (mean ± SD)	14.03 ± 4.04	13.18 ± 4.31	0.044

*Chi-square test or Student's *t*-test.

history of cerebrovascular disease. Venous blood samples were taken from each subject. The levels of total cholesterol, triglyceride, uric acid, and homocysteine (Hcy) were analyzed using routine blood and biochemistry tests.

Two experienced neuroradiologists, who were blinded to all clinical information, reviewed angiograms for each participant. A 4-vessel diagnostic arteriography using the transfemoral approach was performed in all patients to assess complete vessel conditions and collateral circulation. The percentage of vessel stenosis was evaluated by this method as the ratio of the lumen diameter of the residual vessel at the site of the stenosis to the diameter of the vessel distal to the stenosis, according to the North American Symptomatic Carotid Endarterectomy Trial methods for evaluation of internal carotid artery stenosis [26]. A narrowing of blood vessel diameter of 50% or more was regarded as abnormal using DSA. Arteriograms were used to divide patients into extra cranial (EC) and intra cranial (IC) groups. Both EC and IC groups were further categorized according to the location of their abnormal arterial. Collaterals in the anterior circulation were classified into two groups: poor (if none or minimal leptomeningeal anastomoses were visualized and no or minimal filling of the occluded vessel territory occurred) and good (if leptomeningeal anastomoses filled the occluded vessel territory by more than a half). Collaterals in the posterior circulation were considered good when ante grade or reversed unilateral or bilateral filling of the superior cerebellar arteries was considered as poor, when such filling was absent [27].

Genetic analyses

Genomic DNA was extracted from venous blood samples using a commercial DNA extraction kit (TIANGEN, affiliated to QIAGEN, Beijing, China). PCR amplification was performed using *ALDH2* primers (5'-GTCAACTGCTATGATGTGTTTGG-3' and 5'-CCACCAGCAGACCCTCAAG-3') in a 50 μL PCR mixture containing 2 μL DNA template, 10 μL primers, and 1 U *Taq* DNA polymerase for an initial denature step of 94°C for 3 min and then 35 cycles of 94°C for 45 s, 60°C for 30 s and 72°C for 45 s and final extension of 72°C for 5 min. The PCR products were then purified using a commercial kit (TIANGEN) and were directly sequenced by Invitrogen Corporation (Nanjing, China).

Statistical analysis

All statistical analyses were performed using the SPSS 19.0 statistical package (SPSS Inc., Chicago, IL, USA). An x^2-test was used to determine whether the ALDH2 genotypes were in Hardy-Weinberg equilibrium. Differences between groups for continuous values were calculated using Student's *t*-test. For dichotomous variables, the chi-square (x^2) test and logistic regression analysis were applied. The criterion for statistical significance was a *p-value*<0.05.

Results

Characteristics of studied population

In this study, we enrolled 394 patients with MRI-confirmed acute ischemic stroke and 406 gender- and ethnicity-matched healthy con-

Table 2. *ALDH2* polymorphism in patients with cerebral infarction and healthy controls

Genotype	Patients (n=394) n (%)	Controls (n=406) n (%)	*P* value*
GG	254 (64.5)	264 (65.0)	
GA	120 (30.5)	128 (31.5)	
AA	20 (5.1)	14 (3.4)	0.71
Allele frequencies			
G	628 (79.7)	656 (80.8)	
A	160 (20.3)	156 (19.2)	0.69

*Chi-square test.

trols. The median age of patients and controls was 60.21 and 60.99, respectively (*P*>0.05). The number of patients exhibiting a family history of cerebrovascular disease (*P*=0.033), diabetes mellitus (*P*=0.034), homocysteine (*P*=0.044), and total cholesterol (*P*=0.033) were compared to the controls (**Table 1**).

ALDH2 genotypes of patients and controls and association with cerebrovascular stenosis using digital subtraction angiography

The data of genotype (Supplementary Table) generated showed that the frequency of the *ALDH2* genotype did not differ between patients and controls (*P*=0.71). The allele frequency of *ALDH2* in stroke patients was not significantly different from the controls (*P*=0.69) (**Table 2**).

Furthermore, a diagnostic arteriography of the cerebral arteries was conducted in all 394 patients. We found that 366 patients had abnormal cerebral arteries and 28 patients had normal cerebral arteries. We performed subgroup analyses for the DSA abnormalities (EC subtype, IC subtype, EC and IC subtype) after taking into account adjustments for the patients' conventional risk factors (age, male gender, hypertension, diabetes mellitus, family history of cerebrovascular disease, high level of cholesterol, triglyceride, uric acid, or homocysteine). There was a significantly lower frequency of the ALDH2 AA genotype in patients with IC abnormalities (*P*=0.03, *OR 0.18*, 95% CI 0.04-0.81). However the frequency of AA genotype was significantly higher in patients with abnormal ECDSA (*P*=0.03, *OR 6.05*, 95% CI 1.16-31.53). The A allele was not significantly associated with the location of abnormal DSA in these patients (**Table 3**).

Association of ALDH2 genotypes with cerebral collateral circulation

We divided the patients into good and poor collateral vessel subgroups using diagnostic arteriography of the cerebral arteries. These subgroups were correlated with *ALDH2* genotypes after adjustment for confounding factors. We found that there was significant difference between these two subgroups of patients in relation to the distribution of ALDH2 genotypes. The rate of good collateral vessels was significantly lower in the mutation group (GA+AA; *P*=0.001, *OR 3.00*, 95% CI 1.54-5.82). The allele frequency was also significantly different between these two subgroups (*P*=0.02, *OR 1.86*, 95% CI 1.09-3.16; **Table 4**).

Discussion

ALDH2 is ubiquitously expressed in all tissues, such as the heart and brain [13, 14]. The *ALDH2*2* variant has been implicated in various human diseases [18-21] and is associated with the severity of carotid and coronary atherosclerosis [22, 23]. The current study assessed *ALDH2*2* genotypes and allele frequencies and the association with ischemic stroke and cerebral vascular stenosis in a Chinese Han population. Our data showed that *ALDH2* genotypes and allele frequency were not significantly different in stroke patients compared to the controls. However, we did find a significantly lower frequency of patients with the *ALDH2* AA genotype in patients with IC abnormalities. The frequency of the AA genotype was significantly higher in patients with abnormal ECDSA. We also found that the rate of "good" collateral vessels was significantly lower in GA+AA genotypes and in A allele genotypes. Our current study indicates that ALDH2*2 could be a protective factor for IC arteries and a destructive factor for EC arteries in patients with ischemic stroke in this Han Chinese population. Further research with a larger sample size will be required to confirm our current data and facilitate an investigation into the mechanism by which aberrant ALDH2 expression contributes to cerebral atherosclerosis or ischemic stroke.

To date, there have been relatively few association studies [22, 28-30] investigating the association between *ALDH2* polymorphisms and

Table 3. Association of ALDH2 polymorphism with location of DSA abnormalities in patients with cerebral infarction

	EC and IC, n (%)					IC, n (%)					EC, n (%)				
	Abnormal	Normal	β	OR, 95% CI	P*	Abnormal	Normal	β	OR, 95% CI	P	Abnormal	Normal	β	OR, 95% CI	P
GG	68 (60.7)	186 (66.0)				186 (64.6)	68 (64.2)				116 (61.1)	138 (67.6)			
GA	38 (33.9)	82 (29.0)	0.37	1.39 (0.68-2.82)	0.36	96 (33.3)	24 (22.6)	0.34	1.41 (0.65-3.05)	0.39	58 (30.5)	62 (30.4)	0.21	1.24 (0.64-2.38)	0.53
AA	6 (5.4)	14 (5.0)	0.52	1.65 (0.36-7.56)	0.51	6 (2.1)	14 (13.2)	-1.72	0.18 (0.04-0.81)	0.03	16 (8.4)	4 (2.0)	1.80	6.05 (1.16-31.53)	0.03
Allele frequencies															
G	174 (77.7)	454 (80.5)				468 (81.3)	160 (75.5)				290 (76.3)	338 (82.8)			
A	50 (22.3)	110 (19.5)	0.002	1.00 (0.36-2.78)	0.99	108 (18.8)	52 (24.5)	-0.30	0.74 (0.42-1.29)	0.29	90 (23.7)	35 (17.2)	0.51	1.67 (0.99-2.82)	0.06

*Logistic regression analysis.

Table 4. Association of *ALDH2* polymorphism with collateral vessels in patients with cerebral infarction

	Poor n (%)	Good n (%)	β	OR (95% CI)	P*
GG	122 (56.5)	132 (74.2)			
GA+AA	94 (43.5)	46 (25)	1.10	3.00 (1.54-5.82)	0.001
Allele frequencies					
G	332 (76.9)	296 (83.1)			
A	100 (23.1)	60 (16.9)	0.62	1.86 (1.09-3.16)	0.02

*Logistic regression analysis.

cerebrovascular diseases. Similarly, the association between *ALDH2* polymorphisms and the location of collateral cerebrovascular infarction has not been investigated. Our current study reveals that although the ALDH2 504G/A polymorphism is not associated with ischemic stroke, it is associated with the stenosis location of cerebral vascular stenosis. It is especially associated with the occurrence of collateral circulation. In this study, we utilized digital subtraction angiography (DSA) to evaluate cerebral vascular lesions. We excluded miscellaneous factors, such as heavy alcohol drinking or concomitant serious medical illness. This facilitated the generation of reliable data to demonstrate associations with *ALDH2* polymorphisms. However, a previous study conducted by Yao et al. showed that the *ALDH2*1/*2* genotype was a negative risk factor for those displaying high alcohol consumption in stroke patients [29]. Another study showed that there was no significant association between the ALDH2 genotype and the presence of lacunar infarction in Japanese individuals. Interestingly, the ALDH2*1/*1 genotype was associated with a larger number of lesions in Japanese males with lacunar infarction [28]. Guo et al. also suggested that activation of the ALDH2 pathway may serve as a useful index in the identification of stroke-prone subjects and that the ALDH2 pathway may be a potential target of therapeutic intervention of stroke patients [30]. However, to the best of our current knowledge, there has been no association study of ALDH2 polymorphisms between cerebral infarction and healthy controls.

After subdividing DSA abnormalities into EC and IC subtypes, we observed a significantly lower frequency of the *ALDH2* AA genotype in patients with abnormal ICDSA. However, the frequency of the AA genotype was significantly higher in patients with abnormal ECDSA (following adjustment of the patients' conventional risk factors). The data from the IC subtype was similar to that of our previous study [24]. However, the reason for the opposing data in these two subtypes was not clear. Previous studies have shown that African American, Chinese, and Japanese patients tend to display intracranial stenosis, whereas Caucasians tend to display extracranial lesions [31]. Therefore, racial differences might help to explain the latter. In addition, previous studies demonstrated that expression of the ALDH2 enzyme facilitated stroke protection due to reduced 4-HNE levels [29], while the ALDH2 enzyme also facilitated amelioration of chronic alcohol ingestion-induced apoptosis in the cerebral cortex [30, 32, 33]. These data also indirectly showed the role of ALDH2 in regulation of carotid and coronary atherosclerosis.

Furthermore, although a number of noninvasive tools, such as color doppler sonography (CDS), magnetic resonance angiography (MRA), and computerized tomographic angiography (CTA) had been used to evaluate patients with ischemic strokes, cerebral DSA was the gold standard in the identification and severity quantification of atherosclerotic stenosis [34]. Currently, DSA is also considered as the standard reference for the diagnosis of cerebral vascular disease. This is due to its superb spatial and contrast resolution when depicting the vessels, as well as its ability to reveal temporal information on ante grade and collateral flow [35]. This study used DSA to evaluate cerebrovascular lesions and showed that the rate of good collateral vessels was significantly lower in the *ALDH2* GA+AA genotype and A allele frequency compared to that of poor collateral vessels patients. Collateral status has been demonstrated to correlate with acute and final infarct volume and infarct volume expansion in acute ischemic stroke patients [36, 37]. Factors were demonstrated to influence brain collateral circulation were vascular anomalies, risk factors, and blood vessels diameter and associated pressure [38-40]. The collateral circulation is likely to facilitate estimation of the severity of arterial stenosis. The data generated from this study

indicates the potential use of the *ALDH2* GA +AA genotype to predict stenosis severity. The *ALDH2* GA+AA genotype might affect angiogenesis and collateral circulation. Further studies are required to confirm this.

This study has some limitations. For example, the sample size is small and might affect the reliability of the results. Additionally, although we found the association of *ALDH2* polymorphisms with cerebral vessels, we did not discover the underlying mechanism.

Conclusion

In summary, we found that after the adjustment for confounding factors, a significantly lower ALDH2 AA genotype frequency was observed in patients with IC abnormalities along with a higher frequency in patients with EC abnormalities. Furthermore, the rate of good collateral vessels was significantly lower in the mutation group (GA+AA) and genotypes with the A allele.

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Disclosure of conflict of interest

None.

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