Homozygous *ALDH2*2* Is an Independent Risk Factor for Ischemic Stroke in Taiwanese Men

Yueh-Feng Sung, MD, PhD; Chun-Chung Lu, MS; Jiunn-Tay Lee, MD; Yi-Jen Hung, MD; Chaur-Jong Hu, MD; Jiann-Shing Jeng, MD, PhD; Hung-Yi Chiou, PhD; Giia-Sheun Peng, MD, PhD

Background and Purpose—The **2* allele of the aldehyde dehydrogenase 2 gene *(ALDH2)* is the most common variant in Asian populations. The variant resulting in enzyme dysfunction was highly related to coronary artery disease. Recently, genome-wide association studies also discovered that the 12q24 locus near *ALDH2* gene was associated with hypertension and ischemic stroke. This study intended to further investigate whether the above variant of *ALDH2* increases the risk for ischemic stroke in Taiwanese.

Methods—A case–control study was conducted on 914 patients with acute ischemic stroke and 746 nonstroke controls. Polymerase chain reaction and sequencing were used to identify the *ALDH2* genotype. Vascular risk factors, stroke subtypes, vascular stenosis, and stroke outcomes were analyzed.

Results—*ALDH2* genotypes differed significantly between male controls (**1/*1* versus **1/*2* versus **2/*2*=53.8% versus 39.9% versus 6.4%) and male patients with ischemic stroke (**1/*1* versus **1/*2* versus **2/*2*=51.5% versus 37.3% versus 11.2%; *P*=0.048). No significant difference was found between groups for female patients (*P*=0.228). Multivariate logistic regression analysis revealed that the *ALDH2*2/*2* genotype was an independent risk factor for ischemic stroke in male patients (odds ratio, 1.93 [95% confidence interval, 1.07–3.46]; *P*=0.028). Further analysis of men with ischemic stroke demonstrated that the polymorphism of *ALDH2* was not related to vascular risk factors, severity of vascular atherosclerosis, stroke subtypes, and stroke functional outcomes.

Conclusions—The study demonstrated that *ALDH2*2/*2* may be an independent risk factor for ischemic stroke in Taiwanese men, but not in Taiwanese women. **(*Stroke*. 2016;47:2174-2179. DOI: 10.1161/STROKEAHA.116.013204.)**

Key Words: aldehyde dehydrogenase ■ genetic association studies ■ men ■ risk factors ■ Taiwan

Stroke is the leading cause of morbidity and mortality in Taiwanese adults.[1] Because it has multifactorial causes,[2] identification of its underlying pathogenesis and risk factors is essential.[3,4] As a part of the phase I oxidizing enzyme aldehyde dehydrogenase (ALDH) superfamily, ALDH2 is responsible for both ethanol metabolism and biogenic and xenogenic aldehyde detoxification.[5,6] ALDH2 activity deficiency has been reported to increase the risk of cardiovascular disorders.[7,8] Recent genome-wide association studies also discovered that the 12q24 locus near *ALDH2* gene was associated with hypertension and ischemic stroke.[9–11] However, it is essential to have an independent study to test the above findings in different populations with ischemic stroke.

Genetically, the *ALDH2*1* allele is the wild-type, whereas the *ALDH2*2* allelic variant is caused by a single-nucleotide point mutation (G to A) that encodes a glutamate-to-lysine substitution at position 487 (E487K). The latter encodes a partial dominant variant subunit and dramatically reduces ALDH2 tetramer enzyme activity. Individuals with *ALDH2*1/*2* genotype have significantly reduced enzyme activity, whereas individuals with *ALDH2*2/*2* genotype have an almost nil enzyme activity.[12] Notably, both genotypes cause impaired ethanol metabolism[13] and cytoprotection.[6,14] About different populations, the variant *ALDH2*2* allele is far more prevalent in East Asians (30%–50%) than in whites (<5%).[15–17] Statistically, this should be the most common

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From the Graduate Institute of Medical Sciences, National Defense Medical Center, Taipei, Taiwan (Y.-F.S., C.-C.L., G.-S.P.); Department of Neurology (Y.-F.S., J.-T.L., G.-S.P.) and Division of Endocrinology and Metabolism, Department of Internal Medicine (Y.-J.H.), Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; Department of Neurology, College of Medicine, Taipei Medical University and Shuang Ho Hospital, Taiwan (C.-J.H.); Stroke Center and Department of Neurology, National Taiwan University Hospital, Taipei, Taiwan (J.-S.J.); School of Public Health, College of Public Health and Nutrition, Taipei Medical University, Taiwan (H.-Y.C.); and Division of Neurology, Department of Internal Medicine, Taipei Veterans General Hospital, Hsinchu Branch, Hsinchu County, Taiwan (G.-S.P.).

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Correspondence to Giia-Sheun Peng, MD, PhD, Division of Neurology, Department of Internal Medicine, Taipei Veterans General Hospital, Hsinchu Branch. No. 81, Sec. 1, Zhongfeng Rd, Zhudong Township, Hsinchu County 310, Taiwan, E-mail tsghpeng@gmail.com or Hung-Yi Chiou, PhD, School of Public Health, College of Public Health and Nutrition, Taipei Medical University, No. 250, Wu-Hsing St, Taipei 110, Taiwan, E-mail hychiou@tmu.edu.tw

human enzymopathy, as an estimated 560 million East Asians carry the variant *ALDH2*2* allele.[5]

Evidence indicates that those with the *ALDH2*2* allele are prone to gastrointestinal tract malignancies[5,18] and acute coronary syndrome.[7,8,19] Although the relationship between the *ALDH2*2* allele and stroke-related problems remains obscure, correlations with stroke types[20] and severity of atherosclerosis[21] have been reported. This study investigated the association of *ALDH2* gene polymorphisms with the factors related to ischemic stroke in a Taiwanese population.

Methods

Patients and Patient Consents

The study included 914 patients with acute ischemic stroke, from 2 academic medical centers (Tri-Service General Hospital and National Taiwan University Hospital) and 2 community hospitals (Taipei Medical University Hospital and Taipei Medical University-Shuang Ho Hospital), also included were 746 nonstroke control subjects from the Departments of Internal Medicine and Neurology at Tri-Service General Hospital. This study was approved and performed under the ethical guidelines issued by the institutional ethics committees of the participating hospitals, and all patients gave their written informed consent.

Demographic Features and Clinical Characteristics

Demographic features and risk factors were recorded using a structured checklist that included age, body mass index, hypertension, diabetes mellitus (DM), hypercholesterolemia, coronary artery disease, atrial fibrillation, smoking, alcohol drinking, and previous stroke as defined in the Taiwan Stroke Registry.[22] Renal function impairment was defined as serum creatinine >1.2 mg/dL. Liver function impairment was defined as aspartate transaminase or alanine transaminase >40 IU/L. Brain magnetic resonance imaging or computed tomographic scan was performed to confirm the diagnosis of acute ischemic stroke. Acute ischemic strokes were classified into 5 subtypes, including large artery atherosclerosis, small-vessel occlusion, cardioembolism, specific cause, and undetermined cause, based on the criteria of TOAST (Trial of ORG 10172 in Acute Stroke Treatment).[23] The tools for evaluating vascular stenosis included color-coded duplex ultrasonography and magnetic resonance angiography. The grade of stenosis for cervicocerebral vessels was classified into 2 groups: 0% to 49% and ≥ 50%. Extracranial carotid vessels included the common carotid and internal carotid arteries on both sides, whereas intracranial vessels included both the anterior and posterior circulation. Outcomes at 1, 3, and 6 months after the stroke were recorded and represented as modified Rankin Scale scores. A poor outcome was defined as an modified Rankin Scale score of >2.[2]

DNA Collection and Genotyping

DNA was extracted from leukocytes, and the allelotypes of *ALDH2* were determined by multiplex polymerase chain reaction–amplified product length polymorphism analysis as described in Lai et al.[24] Genotyping was performed by laboratory technicians blinded to the case–control status. For quality control and validation purposes, we repeated genotyping on 10% of the samples. The concordance rate for replicate samples was 100%.

Statistical Analysis

Continuous variables were presented as means±SDs, whereas categorical variables were expressed as numbers and percentages. Univariate and multivariate logistic regressions were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between ischemic stroke and any of the confounds. Regression analysis was used for testing the interactions between *ALDH2* genotypes and alcohol drinking in stroke risk. χ^2 and 1-way ANOVA tests were used to analyze the associations between variant *ALDH2* genotypes and clinical characteristics. Statistical analyses were performed with SPSS version 19 software (SPSS, Chicago, IL). Values were considered statistically significant at $P<0.05$.

Results

Table 1 shows that all vascular risk factors were significantly different between the nonstroke and ischemic stroke groups, except for body mass index in both male and female patients, and except for coronary artery disease and alcohol drinking in

Table 1. Clinical Characteristics of Male and Female Nonstroke Controls and Patients With Ischemic Stroke

	Male			Female		
	Nonstroke	Stroke	*P* Value	Nonstroke	Stroke	*P* Value
No. of subjects, n	346	598		400	316	
Age, y	57.2±12.8	63.1±12.0	<0.001	59.1±11.4	68.1±12.6	<0.001
BMI, kg/m^2	25.1±3.4	25.1±3.7	0.955	24.8±4.1	25.0±4.3	0.703
HTN	140 (40.5)	447 (74.7)	<0.001	167 (41.8)	237 (75.0)	<0.001
DM	129 (37.3)	270 (45.2)	0.018	136 (34.0)	140 (44.3)	0.005
H/C	143 (41.3)	291 (48.7)	0.029	188 (47.0)	175 (55.4)	0.026
CAD	23 (6.6)	60 (10.0)	0.077	18 (4.5)	31 (9.8)	0.005
AF	7 (2.0)	50 (8.4)	<0.001	7 (1.8)	58 (18.4)	<0.001
Smoking	157 (45.4)	414 (69.2)	<0.001	15 (3.8)	30 (9.5)	0.002
Alcohol drinking	117 (33.8)	177 (29.6)	0.178	41 (10.3)	7 (2.2)	<0.001
ALDH2 genotype, %			0.048			0.228
**1/*1* vs **1/*2* vs **2/*2*	53.8/39.9/6.4	51.5/37.3/11.2		50.8/41.3/8.0	52.5/36.4/11.1	

Values for age and BMI are expressed as means±SD. Numbers in parentheses are percentages. Statistically significant differences were determined using the χ^2 test for categorical variables, and Student *t* test for continuous variables, between different groups. AF indicates atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; DM, diabetes mellitus; H/C, hypercholesterolemia; and HTN, hypertension.

male patients. Sex differences in the patients with stroke were found: female patients with stroke were 5 years older, had a higher prevalence of atrial fibrillation, and had less smoking and drinking behaviors than their male counterparts. These findings are comparable to a previous report.[25] There was a statistically significant difference in the 3 *ALDH2* genotypic groups between male nonstroke controls and male patients with ischemic stroke (P=0.048); this difference was not present in female patients (P=0.228). To evaluate other inheritance models, 1 model was that the subjects were divided into 2 groups *ALDH2*2/*2* versus *ALDH2*1/*1* and **1/*2* for comparing the difference of the *ALDH2* polymorphism between the groups of stroke and nonstroke. The risk of *ALDH2*2/*2* for ischemic stroke remained significantly increased in male patients, but not in female patients (P=0.014 for male group; P=0.161 for female group). The other model with 2 groups *ALDH2*1/*2* and **2/*2* versus *ALDH2*1/*1*, the group of *ALDH2*1/*2* and **2/*2* did not increase the stroke risk in both sexes (P=0.504 for male group; P=0.636 for female group; result not shown).

Univariate analysis showed that age (OR, 1.04 [95% CI, 1.03–1.05]; P<0.001), hypertension (OR, 4.36 [95% CI, 3.28–5.78]; P<0.001), DM (OR, 1.39 [95% CI, 1.06–1.82]; P=0.019), hypercholesterolemia (OR, 1.35 [95% CI, 1.03–1.76]; P=0.030), atrial fibrillation (OR, 4.42 [95% CI, 1.98–9.86]; P<0.001), smoking (OR, 2.71 [95% CI, 2.06–3.56]; P<0.001), and *ALDH2*2/*2* (OR, 1.84 [95% CI, 1.10–3.08]; P=0.020) were all significant risk factors for ischemic stroke in male patients (Table 2). After adjusting for other confounds for stroke (model A), the risk attributable to the *ALDH2*2/*2* genotype for ischemic stroke remained statistically significant in male patients (OR, 1.93 [95% CI, 1.07–3.46]; P=0.028). Further regression analysis using the interaction of *ALDH2* genotypes and alcohol drinking as a new covariate (model B) demonstrated that the risk attributable to the *ALDH2*2/*2* genotype for ischemic stroke became more statistically significant (OR, 2.47 [95% CI, 1.27–4.82]; P=0.008). A stratified analysis by alcohol drinking revealed that the stroke risk of *ALDH2*2/*2* genotype was higher in nondrinkers (OR, 2.47 [95% CI, 1.27–4.82]; P=0.008) than in drinkers (OR 1.12 [95% CI, 0.31–4.02], P=0.867; Table I in the online-only Data Supplement).

Table 3 compared the clinical characteristics in male patients with ischemic stroke across the various *ALDH2* genotypes. The mean age was younger in the *ALDH2*1/*1* group (61.6±11.6) than in the *ALDH2*1/*2* group (64.8±12.1; P=0.007), and alcohol drinking was significantly lower in the *ALDH2*2/*2* group (P<0.001). There were no other statistically significant differences for vascular risk factors, extracranial carotid stenosis, intracranial stenosis, stroke subtypes, or stroke outcomes in male patients with ischemic stroke with differing *ALDH2* genotypes. Moreover, the male patients with stroke, the *ALDH2*1/*1* genotype, and a history of alcohol drinking were significantly younger (60.0±11.5 years) than those of nondrinkers (62.9±11.6 years; P=0.023; data not shown). After adjusting for the factor of alcohol drinking, there was no significant age difference between *ALDH2* polymorphisms.

Discussion

The present study revealed that the *ALDH2*2/*2* genotype was an independent risk factor for ischemic stroke in Taiwanese men, but not in Taiwanese women. *ALDH2* polymorphism in patients with stroke was associated with drinking behavior, mainly in patients with *ALDH2*1/*1* genotype, but had no association with other vascular risk factors, cervicocerebral stenosis, ischemic stroke subtypes, or stroke outcomes. Notably, the *ALDH2*2/*2* genotype increased the risk of ischemic stroke independently of conventional vascular risk factors, so the underlying pathophysiology of this risk remains unclear.

ALDH2 is well-known in ethanol metabolism and is an important determinant for drinking behavior, so past reports have suggested that the risk of stroke related to *ALDH2* polymorphism is dependent on the interaction between its phenotype and lifestyle or environment.[1,26] Subjects with the *ALDH2*2* allele have low

Table 2. Univariate and Multivariate Logistic Regression Analysis for Risk Factors in Men With Ischemic Stroke

	Univariate			Multivariate (Model A)*			Multivariate (Model B)†		
Variable	OR	95% CI	*P* Value	OR	95% CI	*P* Value	OR	95% CI	*P* Value
Age, y	1.04	1.03–1.05	<0.001	1.04	1.03–1.05	<0.001	1.04	1.02–1.05	<0.001
HTN	4.36	3.28–5.78	<0.001	4.08	2.98–5.57	<0.001	4.07	2.97–5.58	<0.001
DM	1.39	1.06–1.82	0.019	1.12	0.82–1.53	0.460	1.13	0.82–1.54	0.457
H/C	1.35	1.03–1.76	0.030	1.37	1.01–1.87	0.045	1.36	1.00–1.86	0.054
AF	4.42	1.98–9.86	<0.001	4.07	1.73–10.40	0.002	4.10	1.66–10.12	0.002
Smoking	2.71	2.06–3.56	<0.001	3.81	2.73–5.30	<0.001	3.79	2.72–5.29	<0.001
Alcohol drinking	0.82	0.62–1.09	0.178	0.70	0.49–0.99	0.043	1.02	0.66–1.58	0.925
ALDH2									
**1/*1*	1.00‡			1.00‡			1.00‡		
**1/*2*	0.98	0.74–1.29	0.864	0.81	0.59–1.13	0.219	1.12	0.76–1.65	0.581
**2/*2*	1.84	1.10–3.08	0.020	1.93	1.07–3.46	0.028	2.47	1.27–4.82	0.008

AF indicates atrial fibrillation; CI, confidence interval; DM, diabetes mellitus; H/C, hypercholesterolemia; HTN, hypertension; and OR, odds ratio.

*Model A: statistically significant differences were determined using multiple logistic regression after adjusting for the other factors.

†Model B: the interaction of alcohol drinking and *ALDH2* genotypes was constructed as a covariate in multiple logistic regression.

‡Reference value.

Table 3. Clinical Characteristics of Male Patients With Ischemic Stroke Across Different *ALDH2* Genotypes

	*1/*1	*1/*2	*2/*2	*P* Value
No. of subjects, n	308	223	67	
Age, y	61.6±11.6	64.8±12.1	65.0±12.0	0.007*, 0.096†
HTN	233 (75.6)	168 (75.3)	46 (68.7)	0.475
DM	140 (45.5)	98 (43.9)	32 (47.8)	0.849
H/C	159 (51.6)	104 (46.6)	28 (41.8)	0.258
CAD	34 (11.0)	18 (8.1)	8 (11.9)	0.457
AF	28 (9.1)	18 (8.1)	4 (6.0)	0.691
RFI‡	86 (27.9)	63 (28.3)	20 (29.9)	0.951
LFI§	46 (14.9)	13 (13.9)	13 (19.4)	0.542
Smoking	224 (72.7)	145 (65.0)	45 (67.2)	0.153
Alcohol drinking	138 (44.8)	33 (14.8)	6 (9.0)	<0.001
Previous stroke	85 (27.6)	63 (28.3)	17 (25.4)	0.899
ECS (≥50%)	26 (8.4)	23 (10.3)	6 (9.0)	0.760
ICS (≥50%)	107 (34.7)	74 (33.2)	26 (38.8)	0.696
TOAST classification				0.938
Large vessel	110 (35.7)	80 (35.9)	25 (37.3)	
Small vessel	102 (33.1)	65 (29.1)	19 (28.4)	
Cardioembolism	25 (8.1)	17 (7.6)	5 (7.5)	
Specific	7 (2.3)	7 (3.1)	3 (4.5)	
Undetermined	64 (20.8)	54 (24.2)	15 (22.4)	
mRS>2,‖ mo				
1	87 (31.0)	69 (33.0)	23 (35.9)	0.717
3	68 (25.2)	48 (24.0)	20 (31.3)	0.505
6	56 (22.0)	43 (22.8)	16 (26.2)	0.775

Values are mean±SD. Numbers in parentheses are percentages. Data were analyzed by χ^2 and 1-way-ANOVA tests as appropriate. AF indicates atrial fibrillation; CAD, coronary artery disease; DM, diabetes mellitus; ECS, extracranial carotid stenosis; H/C, hypercholesterolemia; HTN, hypertension; ICS, intracranial stenosis; LFI, liver function impairment; mRS, modified Rankin Scale; RFI, renal function impairment; and TOAST, Trial of ORG 10172 in Acute Stroke Treatment.

* **1/*1* vs. **1/*2*.

† **1/*1* vs. **2/*2*.

‡RFI: creatinine >1.2 mg/dL.

§LFI: aspartate transaminase >40 IU/L or alanine transaminase >40 IU/L.

‖The data were collected only from patients with continuous follow-up.

enzyme activity that impairs acetaldehyde metabolism.[16] The resulting acute acetaldehyde accumulation induces strongly negative psychophysiological reactions, as well as cardiovascular responses, during low to moderate alcohol intake.[15,27,28] The unpleasant effects may lead to alcohol avoidance and may significantly reduce the prevalence of alcoholism in carriers of the *ALDH2*2* allele; this may also indirectly reduce the detrimental influence of ethanol on cardiovascular risk.[29] Yao et al[17] first reported that the frequency of the *ALDH2*2* allele in Taiwanese patients with stroke who were also heavy drinkers was significantly lower than those who were not heavy drinkers. The low incidence of the *ALDH2*2* allele among heavy drinkers may be directly related to the tendency of Taiwanese patients who have the allele to live an alcohol-avoidant lifestyle.[29] On the contrary, a recent report indicated that if subjects with the *ALDH2*2* allele and vascular risk factors ignore this inborn alcohol avoidance and engage in binge drinking, such drinking behavior may increase the risk of stroke.[15,24,28] Notably, these previous studies indicated that *ALDH2* polymorphism as a risk indicator for stroke was because of its high correlation with drinking behavior.

Despite the above research, whether *ALDH2* polymorphism is independent of alcohol drinking behavior as a risk factor for ischemic stroke remained unclear. A recent, well-designed animal study demonstrated that ALDH2 protects against stroke by detoxification of 4-hydroxynonenal and activation of the protein kinase C pathway for neuroprotection.[30] Interestingly, increasing evidence from the genome-wide association studies discovered that 2 loci near *ALDH2* gene are related to cardiovascular disorders. One, the 12q24.13 locus, was strongly

associated with elevated blood pressure with ethnic specificity.[10] Another, the 12q24.12 locus, was first identified as a novel association with all ischemic stroke subtypes and was not mediated by conventional cardiovascular risk factors.[9] However, a recent study demonstrated that the 12q24 locus was specifically associated with the small artery occlusion stroke subtype, rather than for all stroke subtypes.[11] These data highlight the potential involvement of the *ALDH2* gene in the risk of ischemic stroke. Together, the collected data indicated that the ALDH2 pathway may play an important role in stroke pathogenesis and may be a potential target of therapeutic intervention in the future. However, there is still a lack of strong evidence that can clarify the relationship between the *ALDH2*2* allele and stroke.

A community study in Japan had reported that the *ALDH2*1/*1* genotype was significantly associated with the prevalence of multiple lacunar infarcts in Japanese men, after comparing this genotype to Japanese men with *ALDH2*1/*2* or *ALDH2*2/*2*.[20] However, when single and multiple lacunar infarcts were combined in the stroke group, the study found no correlation between stroke and *ALDH2* polymorphism. Another recent study[21] of 82 Chinese Han female patients with cerebral infarction indicated that the *ALDH2*2* allele increased the risk of stroke. In this case, although when adjusted for drinking status, the study found no correlation between stroke and *ALDH2* polymorphism. Therefore, no clinical data have conclusively determined whether *ALDH2* polymorphism is an independent risk factor for ischemic stroke.

Table 1 herein demonstrated a sex difference in the association of *ALDH2* polymorphism and the incidence of stroke. The *ALDH2*2/*2* genotype significantly increased the risk of ischemic stroke in Taiwanese male patients, but not in female patients. The potential mechanistic basis of protection against stroke in females may be related to estrogen activation of phosphoinositol-3-kinase and protein kinase C leading to phosphorylation of ALDH2 that decreases toxic aldehydes generated by reactive oxygen species.[31] After adjusting for all other risk factors for stroke, including drinking behavior, by multivariate logistic regression analysis, the *ALDH2*2/*2* genotype was still an independent risk factor for stroke in men, with an OR 1.93 higher than the factors of age (OR, 1.04), DM (OR, 1.12), and hypercholesterolemia (OR, 1.37; Table 2). With regard to the effect of the interaction between the *ALDH2* genotype and alcohol drinking, the risk attributable to the *ALDH2*2/*2* genotype for ischemic stroke became more significant, indicating that alcohol may modify the stroke risk in patients with the *ALDH2*2/*2* genotype. Further analysis of clinical characteristics of Taiwanese male patients with acute ischemic stroke demonstrated that differing *ALDH2* genotypes were not correlated with other risk factors, severity of vascular atherosclerosis, stroke subtypes, or outcomes, except for the *ALDH2*1/*1* genotype, which was correlated with a high prevalence of drinking behavior (Table 3). Thus, this is the first study to demonstrate that the *ALDH2*2/*2* genotype is independent from drinking behavior or other conventional risk factors for ischemic stroke.

Although having the *ALDH2*2/*2* genotype does not guarantee cardiovascular-related morbidity, this inborn risk of cardiovascular diseases may be exacerbated by unhealthy lifestyles or environmental toxins.[14] It is essential to identify the source of the correlation between the *ALDH2*2* allele and stroke risk for the purpose of targeting treatment in the future. The stroke risk discovered only in patients with the *ALDH2*2/*2*, not *ALDH2*1/*2* genotype may be attributable to a total loss of ALDH2 enzyme activity, leading to impairment of detoxification and neuroprotection.[14,30]

Although the underlying mechanism is still unclear, a novel class of molecule was recently found, termed aldehyde dehydrogenase activators, that enhanced ALDH enzyme activity. Of these, Alda-1 [N-(1,3-benzodioxol-5-ylmethyl)-2,6-dichlorobenzamide] was the prototype, identified as enhancing the catalytic activity of ALDH2 in vitro and in vivo.[14,32] The drug was shown to not only significantly increase the catalytic activity of wild-type ALDH2, but also effectively restore the activity of the E487K mutant ALDH2. Furthermore, it also protected ALDH2 enzymatic activity from 4-hydroxynonenal–induced inactivation, even in the presence of a high concentration of 4-hydroxynonenal. Thus, these aldehyde dehydrogenase activators may serve as new therapeutic agents for patients having pathology related to deficient ALDH2 activity.

The strength of this study is that much higher prevalence of the *ALDH2*2* allele in our population (and Asians in general), when compared with the European whites, allowed us to investigate the role of *ALDH2* variant and its interaction with alcohol drinking in ischemic stroke. However, our study has several limitations. Although our sample size was larger than those of previous similar studies, further studies with a larger sample size are necessary to confirm our findings. Second, patient selection biases must be considered. In this study, we recruited most of the nonstroke control subjects from the Division of Endocrinology and Metabolism and Department of Neurology. The prevalence of vascular risk factors in this nonstroke group was high, especially that of DM. This form of selection bias could explain why DM was not an independent risk factor for ischemic stroke after multivariate analysis (Table 2). Future studies that recruit healthy control subjects can solve this problem. Third, magnetic resonance angiography may overestimate stenosis of the major arteries. Conventional digital subtraction angiography is still the gold standard for confirming cervicocerebral stenosis.

Conclusions

Our study is the first to demonstrate that the *ALDH2*2/*2* genotype is an independent risk factor for ischemic stroke in male patients. The risk of *ALDH2*2/*2* for ischemic stroke was not associated with vascular risk factors, cervicocerebral stenosis, or ischemic stroke subtypes. Based on the encoding an extreme low enzyme activity of this homozygous variant alleles and the independence of the risk, we infer that deficiency of ALDH2 enzyme activity in its metabolic pathway may play a major role in the development of stroke. Our findings may benefit stroke screening and diagnosis and improve prevention strategies, in this particular high-risk population.

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Disclosures

None.

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Homozygous *ALDH2*2* Is an Independent Risk Factor for Ischemic Stroke in Taiwanese Men

Yueh-Feng Sung, Chun-Chung Lu, Jiunn-Tay Lee, Yi-Jen Hung, Chaur-Jong Hu, Jiann-Shing Jeng, Hung-Yi Chiou and Giia-Sheun Peng

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ONLINE SUPPLEMENT

Supplementary Table 1. Univariate and multivariate logistic regression analysis for risk factors in men with ischemic stroke

	Univariate			Multivariate (Model A)[a]			Multivariate (Model B)[b]			Multivariate (Model C)[c]		
Variable	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Age	1.04	1.03-1.05	<0.001	1.04	1.03-1.05	<0.001	1.04	1.02-1.05	<0.001	1.04	1.02-1.05	<0.001
HTN	4.36	3.28-5.78	<0.001	4.08	2.98-5.57	<0.001	4.07	2.97-5.58	<0.001	4.07	2.97-5.58	<0.001
DM	1.39	1.06-1.82	0.019	1.12	0.82-1.53	0.460	1.13	0.82-1.54	0.457	1.13	0.82-1.54	0.457
H/C	1.35	1.03-1.76	0.030	1.37	1.01-1.87	0.045	1.36	1.00-1.86	0.054	1.36	1.00-1.86	0.054
AF	4.42	1.98-9.86	<0.001	4.07	1.73-10.40	0.002	4.10	1.66-10.12	0.002	4.10	1.66-10.12	0.002
Smoking	2.71	2.06-3.56	<0.001	3.81	2.73-5.30	<0.001	3.79	2.72-5.29	<0.001	3.79	2.72-5.29	<0.001
Alcohol drinking	0.82	0.62-1.09	0.178	0.70	0.49-0.99	0.043	1.02	0.66-1.58	0.925			
ALDH2												
**1/*1*	1.00[d]			1.00[d]			1.00[d]					
**1/*2*	0.98	0.74-1.29	0.864	0.81	0.59-1.13	0.219	1.12	0.76-1.65	0.581			
**2/*2*	1.84	1.10-3.08	0.020	1.93	1.07-3.46	0.028	2.47	1.27-4.82	0.008			
ALDH2 x Alcohol drinking												
**1/*1* Alcohol(-) (n=286)							1.00[d]			1.00[d]		
**1/*2* Alcohol(-) (n=287)							-[e]	-[e]	-[e]	1.12	0.76-1.65	0.581
**2/*2* Alcohol(-) (n=77)							-[e]	-[e]	-[e]	2.47	1.27-4.82	0.008
**1/*1* Alcohol(+) (n=208)							-[e]	-[e]	-[e]	1.02	0.66-1.58	0.925
**1/*2* Alcohol(+) (n=74)							0.35	0.17-0.72	0.004	0.40	0.22-0.72	0.002
**2/*2* Alcohol(+) (n=12)							0.44	0.10-1.89	0.270	1.12	0.31-4.02	0.867

[a]Model A: statistically significant differences were determined using multiple logistic regression after adjusting for the other factors

[b]Model B: the interaction of alcohol drinking and *ALDH2* genotypes was constructed as a covariate in multiple logistic regression

[c]Model C: the variables of *ALDH2* genotypes and alcohol drinking were omitted in this Model due to insufficient degrees of freedom for regression

[d]Reference value

[e]The data is unavailable from SPSS version 19 software

Abbreviations: AF, atrial fibrillation; DM, diabetes mellitus; H/C, hypercholesterolemia; HTN, hypertension