Clinical Sciences

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

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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

Stoke is the leading cause of morbidity and mortality in Taiwanese adults. Because it has multifactorial causes, identification of its underlying pathogenesis and risk factors is essential.1,3 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/*1 allele is the wild-type, whereas the ALDH2*2 allele 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.2 Notably, both genotypes cause impaired ethanol metabolism13 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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human enzymopathy, as an estimated 560 million East Asians carry the variant ALDH2*2 allele.\textsuperscript{5}

Evidence indicates that those with the ALDH2*2 allele are prone to gastrointestinal tract malignancies\textsuperscript{5,18} and acute coronary syndrome.\textsuperscript{7,8,39} Although the relationship between the ALDH2*2 allele and stroke-related problems remains obscure, correlations with stroke types\textsuperscript{20} and severity of atherosclerosis\textsuperscript{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.\textsuperscript{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 tomography 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).\textsuperscript{21} 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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsuperscript{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

Values for age and BMI are expressed as means±SD. Numbers in parentheses are percentages. Statistically significant differences were determined using the χ\textsuperscript{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.\(^2\) There was a statistically significant difference in the 3 ALDH2 genotypic groups between male nonstroke controls and male patients with ischemic stroke:\(^*\) (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.\(^1\)\(^\text{2}\) Subjects with the ALDH2*2 allele have low

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate (Model A)*</th>
<th>Multivariate (Model B)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>(P) Value</td>
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<td>Age, y</td>
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<td>1.03–1.05</td>
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<td>HTN</td>
<td>4.36</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td>DM</td>
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<td>0.019</td>
</tr>
<tr>
<td>H/C</td>
<td>1.35</td>
<td>1.03–1.76</td>
<td>0.030</td>
</tr>
<tr>
<td>AF</td>
<td>4.42</td>
<td>1.98–9.86</td>
<td>&lt;0.001</td>
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<td>Smoking</td>
<td>2.71</td>
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<td>Alcohol drinking</td>
<td>0.82</td>
<td>0.62–1.09</td>
<td>0.178</td>
</tr>
</tbody>
</table>

ALDH2

\(\text{*1/*1} \) 1.00†

\(\text{*1/*2} \) 0.98 0.74–1.29 0.864 0.81 0.59–1.13 0.219 1.12 0.76–1.65 0.581

\(\text{*2/*2} \) 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.
enzyme activity that impairs acetaldehyde metabolism. The resulting acute acetaldehyde accumulation induces strongly negative psychophysiological reactions, as well as cardiovascular responses, during low to moderate alcohol intake. 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. Yao et al 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. 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. 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. 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

<table>
<thead>
<tr>
<th>Clinical Characteristics of Male Patients With Ischemic Stroke Across Different ALDH2 Genotypes</th>
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<tr>
<td>**</td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>No. of subjects, n</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>HTN</td>
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<td>Alcohol drinking</td>
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<td>Previous stroke</td>
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<td>3</td>
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<td>6</td>
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</table>

Values are mean±SD. Numbers in parentheses are percentages. Data were analyzed by χ² 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.
associated with elevated blood pressure with ethnic specificity. 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. However, a recent study demonstrated that the 12q24 locus was specifically associated with the small artery occlusion stroke subtype, rather than all stroke subtypes. 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. 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 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. 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. 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.

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. 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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http://stroke.ahajournals.org/content/suppl/2016/08/02/STROKEAHA.116.013204.DC1

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### ONLINE SUPPLEMENT

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate (Model A)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Multivariate (Model B)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Multivariate (Model C)&lt;sup&gt;c&lt;/sup&gt;</th>
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<td>0.62-1.09</td>
<td>0.178</td>
<td>0.70</td>
</tr>
</tbody>
</table>

**ALDH2**

*1/*1 <sup>d</sup>

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

**ALDH2 x Alcohol drinking**

*1/*1 Alcohol(-) (n=286) 1.00<sup>d</sup>

*1/*2 Alcohol(-) (n=287) 1.00<sup>d</sup>

*2/*2 Alcohol(-) (n=77) 1.00<sup>d</sup>

*1/*1 Alcohol(+) (n=208) 1.00<sup>d</sup>

*1/*2 Alcohol(+) (n=74) 1.00<sup>d</sup>

*2/*2 Alcohol(+) (n=12) 1.00<sup>d</sup>

*1/*1 Alcohol(-) (n=286) 1.00<sup>d</sup>

*1/*2 Alcohol(-) (n=287) 1.00<sup>d</sup>

*2/*2 Alcohol(-) (n=77) 1.00<sup>d</sup>

*1/*1 Alcohol(+) (n=208) 1.00<sup>d</sup>

*1/*2 Alcohol(+) (n=74) 1.00<sup>d</sup>

*2/*2 Alcohol(+) (n=12) 1.00<sup>d</sup>

*1/*1 Alcohol(-) (n=286) 1.00<sup>d</sup>

*1/*2 Alcohol(-) (n=287) 1.00<sup>d</sup>

*2/*2 Alcohol(-) (n=77) 1.00<sup>d</sup>

*1/*1 Alcohol(+) (n=208) 1.00<sup>d</sup>

*1/*2 Alcohol(+) (n=74) 1.00<sup>d</sup>

*2/*2 Alcohol(+) (n=12) 1.00<sup>d</sup>

<sup>a</sup>Model A: statistically significant differences were determined using multiple logistic regression after adjusting for the other factors.

<sup>b</sup>Model B: the interaction of alcohol drinking and ALDH2 genotypes was constructed as a covariate in multiple logistic regression.
Model C: the variables of *ALDH2* genotypes and alcohol drinking were omitted in this Model due to insufficient degrees of freedom for regression.

Reference value

The data is unavailable from SPSS version 19 software.

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