East Asian Variant of Aldehyde Dehydrogenase 2 (ALDH2*2) is Associated with Coronary Spastic Angina: Possible Roles of Reactive Aldehydes and Implications of Alcohol Flushing Syndrome

Running title: Mizuno et al.; Coronary Spastic Angina and variant ALDH2*2

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Abstract

**Background**—Coronary spastic angina (CSA) is a common disease among East Asians including Japanese. The prevalence of alcohol flushing syndrome (AFS) associated with deficient activity of variant aldehyde dehydrogenase 2 (ALDH2*2) genotype is prevalent among East Asians. We examined whether CSA is associated with ALDH2*2 genotype in Japanese.

**Methods and Results**—The study subjects consisted of 202 patients in whom intracoronary injection of acetylcholine was performed by angiography on suspicion of CSA (119 men and 83 women, mean age 66.2±11.4). They were divided into CSA (112 patients) and control (90 patients) groups. ALDH2 genotyping was performed by the direct application of the TaqMan polymerase chain reaction system on dried whole blood. Clinical and laboratory data were examined using conventional methods. The frequencies of male gender, ALDH2*2 genotype carriers, AFS, tobacco smoking and the plasma level of uric acid were higher (P < 0.001, P < 0.001, P < 0.001, and P = 0.007, respectively) and the plasma HDL cholesterol levels were lower (P < 0.001) in the CSA group than the control group. The multivariable logistic regression analysis revealed that ALDH2*2 genotype and smoking were significantly associated with CSA (P < 0.001 and P = 0.024, respectively).

**Conclusions**—East Asian variant ALDH2*2 genotypes and hence deficient ALDH2 activity were associated with CSA in Japanese. These data support further investigation of treatment targeting aldehydes for CSA.

**Key words:** alcohol, coronary spastic angina, coronary spasm, ischemic heart disease, alcohol dehydrogenase gene, aldehyde dehydrogenase 2, ALDH2, Aldehyde
Introduction

Coronary spastic angina (CSA) or angina pectoris caused by coronary artery spasm is a common disease among East Asians including Japanese but is rare in the Western populations.\(^1\)-\(^4\) Alcohol (ethanol) flushing syndrome (AFS) including facial flushing, headache, nausea, and palpitation in response to a small amount of alcohol intake is common among East Asians but is almost absent in other populations of the world.\(^5\)-\(^7\) Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase subunit beta (ADH1B) and then to acetic acid by aldehyde dehydrogenase 2 (ALDH2).\(^8\) There are polymorphisms in human \textit{ADH1B} and \textit{ALDH2} genes and the carriers of variant \textit{ADH1B} or \textit{ADH1B*2} (Arg47His) genotypes have an enhanced enzymatic activity, while those of variant \textit{ALDH2} or \textit{ALDH2*2} (Glu504Lys) genotype have a severely reduced enzymatic activity.\(^5\)-\(^7\) These genetic variants are commonly found in East Asians (Chinese, Japanese, Koreans, and Taiwanese) but rare or absent in other ethnic populations of the world.\(^5\)-\(^7\) The carriers of these variant genes manifest AFS on intake of small amounts of alcohol due to accumulation of acetaldehyde.\(^5\)-\(^8\)

We have shown that AFS and alcohol patch test are associated with CSA.\(^9\) We and others have reported that CSA can be induced by alcohol ingestion, particularly in those with AFS.\(^10\),\(^11\) However, the relationships of CSA with \textit{ADH1B} and \textit{ALDH2} gene polymorphisms remain to be elucidated. In the present study, we investigated the association of polymorphisms of \textit{ADH1B} and \textit{ALDH2} with CSA.

Methods

Study subjects

The study subjects consisted of 202 Japanese patients (119 men with a mean age of 64.8 ± 12.5,
and 83 women with a mean age of 68.3 ± 9.3) who had underwent coronary angiography and intracoronary injection of acetylcholine (ACh) on suspicion of CSA because of episodes chest discomfort occurring at rest between January, 2010 and June 2014 at our institution. One hundred twelve patients (79 men and 33 women, mean age 66.3 ± 10.9) were diagnosed as CSA on the basis of angiographically documented coronary spasm and the remaining 90 patients (33 men and 57 women, mean age 66.2 ± 12.0) who had no coronary spasm induced as non-CSA and served as the controls. Patients with acute myocardial infarction, organic stenosis of ≥ 75 %, three-vessel organic disease, left main trunk lesion, uncontrolled arrhythmias, heart failure, resting hypertension > 180/110 mmHg, acute systemic illness, and hepatic or renal insufficiency or other severe conditions were excluded from the study. All vasoactive medications including calcium channel blockers, beta-receptor blockers, angiotensin converting enzyme inhibitors, angiotensin II receptors blockers and statins were withdrawn for at least three days before the angiography except for nitroglycerin used for attacks. The study was approved by the ethics committee of our institution and written informed consent was obtained from each patient.

Angiographic documentation of coronary spasm

Coronary spasm was defined as a transient total or subtotal occlusion or severe diffuse vasoconstriction of an epicardial coronary artery associated with ischemic changes on ECG with or without chest discomfort. The final diagnosis was determined with the consensus of 3 investigators blinded to genotype/flushing phenotype. Coronary spasm was induced by the intracoronary injection of acetylcholine (ACh) (Daiichi-Sankyo Co., Tokyo, Japan) after diagnostic catheterization in the morning.\textsuperscript{12-14} ACh dissolved in 5 ml of warmed 0.9 % saline was infused manually over 20 seconds into the left coronary artery (LCA) in incremental doses of 10, 20, 50 and 100 µg and then 10, 20 and, 50 µg into the right coronary artery (RCA) depending on
the vascular reactivity in 20 seconds under the continuous monitoring of 12 lead ECG and blood pressure with a temporary pacemaker in place in the morning. These doses of ACh corresponded to $2 \times 10^{-6}$ mol/L-$10^{-5}$ mol/L. Coronary angiography was performed 1 min. after each ACh injection or when ischemic ECG changes appeared. Coronary spasm induced by this method usually disappeared spontaneously within 1-2 minutes and both the left and right coronary arteries could be examined separately unless severe spasm occurred in the left coronary artery and necessitated the prompt injection of nitroglycerin (GTN) or isosorbide dinitrate (ISDN) into the artery. Finally, nitroglycerin or isosorbide dinitrate was infused to relieve spasm and examine organic lesions. The specificity of this test for variant angina was 99 %. The spasm arteries were quantitatively evaluated after GTN or ISDN with CAAS II software (PIE Medical Imaging, Maastricht, Limburg, Netherlands) but not in all patients. Significant organic coronary stenosis was defined as > 50 % luminal diameter.

**Genotyping**

The details of the method were reported. Briefly, the SNP genotyping of the ADH1B (Arg47His; rs1229984) and ALDH2 (Glu504Lys; rs671) was performed using the TaqMan assay on ABI 7300 Real Time PCR System (APPLIED BIOSYSTEMS, Foster City, California, USA) without DNA extraction on whole blood. The mixture was 20 μL, and consisted of 10 μL of a Thunderbird Probe qPCR Mix (QPS-101, TOYOBO, Osaka, Japan), 0.4 μL of a 50 × ROX reference dye (TOYOBO), 1 μL of a 20 × ADH1B TaqMan Probe & ADH1B Primer Mix (C_2688467_20, TaqMan® Drug Metabolism Genotyping Assays, ABI) or a 20 × ALDH2 TaqMan Probe & ALDH2 Primer Mix (C_11703892_10, ABI), 2 μL of each PCR product, and 6.6 μL of distilled water. The thermal cycling process was performed according to the Applied Biosystems PCR conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of denaturation at 95 °C.
for 15 s, and annealing and extension at 60 °C for 1 min. The results were analyzed by ABI
Prism 7300 SDS software. The genotyping was performed with the identification of the study
subjects blinded at the Kinoshita Laboratory, School of Pharmaceutical Sciences, Mukogawa
Women’s University.

**Questionnaire survey**

The subjects were asked to fill out a simple questionnaire concerning alcohol flushing on alcohol
intake, alcohol drinking habit, and smoking. Habitual drinker was defined as an alcohol drinker
more than 5 days a week. Alcohol flushing was defined as a current or a history of facial flushing
immediately after drinking a glass of beer (ethanol 10 g) or the equivalent alcoholic beverages.
Smokers were defined as current and past smokers.

**Blood chemistry measurements**

Blood samples for measurement of clinical chemistry and other data were collected after an
overnight fast with the patients in the supine position. The biochemical and other analyses were
done using standard laboratory procedures.

**Statistical analysis**

Allele frequencies were determined by direct gene counting and genotype distributions were
checked for departure from Hardy-Weinberg equilibrium using the Pearson Chi square test. Odds
ratio (OR) was calculated as a measure of association of genotype with CSA under assumptions
of additive, dominant, or recessive mode of inheritance. The baseline clinical data were
expressed as the mean ± SD or median (25th, 75th percentile) for continuous variables and
differences within the group were evaluated with unpaired t-test or the Mann-Whitney rank sum
test. For discrete variables, the data were expressed as counts and percentages and analyzed with
the Chi square test. The correlation between the variables was assessed using Spearman’s rank
correlation coefficient. A multiple logistic regression analysis was performed to determine the risk factors of CSA. Independent variables were included on the basis of theoretical and clinical grounds, the results of a bivariate analysis ($P < 0.05$) and collinearity. A two-tailed value of $P < 0.05$ was considered to be as statistically significant. The analyses were done using the STATA software program (STATA 11.0, STATA Corp., College Station, TX, U.S.A.).

**Results**

**Characteristics of the study patients**

Table 1 compares the clinical characteristics between the CSA and control groups. Male gender, smoker, and plasma uric acid levels were significantly higher ($P < 0.001, P < 0.001, \text{and } P = 0.007$, respectively) and the plasma level of HDL cholesterol and frequency of male habitual alcohol drinker significantly lower ($P<0.001 \text{ and } P=0.016$, respectively) in the CSA group than the control group. The frequency of AFS was significantly higher in the CSA group than in the control group ($68.8 \% \text{ vs. } 41.1 \%, P < 0.001$). Thus, there was a strong association between AFS and CSA [$\text{OR}=3.15, 95 \% \text{ confidence interval (CI), } 1.77-5.63$]. Seventy three (65.2 %), 23 (20.5 %) and 16 (14.3%) patients had zero, one and two vessel disease in the CSA group. Six patients had had a stent implanted. Twelve (10.7%), 18 (16.1%) and 82 (73.2%) patients had total occlusion, subtotal occlusion, and severe diffuse vasoconstriction, respectively, and ST segment elevation appeared in 34 patients (30.0 %) and ST segment depression in the 78 patients (70.0%) in the CSA group. Thus, the majority of the patients in the CSA group had normal or no significant organic stenosis and had attacks of severe vasoconstriction (**Figure 1a and b**). The ACh provocation tests could be done in 191 patients on both the left and right coronary arteries. Six patients had the severe left coronary spasm requiring the prompt injection of nitroglycerin
and the right coronary artery therefore could not be assessed. ACh infusion was not done into the right coronary artery because the artery was hypoplastic in 5 patients. Of the 191 patients in whom ACh was infused both into the LCA and RCA, 66 (34.6%) had spasm in both the LCA and RCA (Figure 1a and b).

**The genotype distribution of ADH1B and ALDH2**

Table 2 shows the number of study subjects by genotype for ADH1B and ALDH2 in the CSA and control groups. The genotype distributions did not depart from the Hardy-Weinberg equilibrium for both ADH1B ($\chi^2=0.301, P=0.58$ for the CSA group and $\chi^2=0.385, P=0.53$ for the control group) and ALDH2 genes ($\chi^2=0.463, P=0.50$ for CSA group and $\chi^2=0.020, P=0.89$ for control group). There was no significant overall difference in genotype distribution for ADH1B*1 between the CSA and control groups ($\chi^2 = 0.484, P = 0.79$) and a large majority of the study patients had variant ADH1B*2 (ADH1B*1/*2 + ADH1B*2/*2) genotypes in both groups (94.4 % vs. 95.5 %, $P = 0.72$) (Table 2). There were likewise no significant differences in the additive, dominant and recessive models of inheritance between the two groups (Figure 2a).

There was a significant overall difference in genotype distribution of ALDH2 between the CSA and control groups ($\chi^2 = 15.15, P < 0.001$). The frequency of ALDH2*2 allele was 32.1 % in the CSA group and 15.6 % in the non-CSA group respectively, with that of the whole study population including both the CSA and non-CSA groups being 24.8 %. Since the allele frequency of ALDH2*2 in the general population was 23.5 % (247/1050) in Kumamoto and 24.3 % (183/752) in Tokyo, Japan, the allele frequency of the CSA group was higher and that of the non-CSA group lower than that of the general population in Japan.

There were significant differences in the additive and dominant models of ALDH2 between the CSA and control groups [OR = 6.40 (95 % CI, 1.34-30.54), $P = 0.021$ and OR =
3.05 (95 % CI, 1.69-5.50), P < 0.001 respectively] (Figure 2b). These finding were consistent with the well-recognized dominant role of the $ALDH2^*2$ genotype in affecting enzymatic activities$^{5-7,17,18}$ and indicated that the genotype existed mainly as the heterozygote $ALDH2^*1^*/2$ (Figure 3a and Figure 3b). We therefore combined heterozygote ($ALDH2^*1^*/2$) and homozygote ($ALDH2^*2^*/2$) as a single category of $ALDH2^*2$ or $ALDH2^*2$ carriers and compared them with the wild homozygote $ALDH2^*1^*/1$ in the analyses.

Table 3 compares the clinical characteristics between the wild $ALDH2^*1$ genotype and the variant genotype $ALDH2^*2$ groups. The frequencies of CSA and AFS were significantly higher in the $ALDH2^*2$ group than in the $ALDH2^*1^*/1$ group [70.5 % vs. 43.9 %, OR = 3.05 (95 % CI, 1.69-5.50), P < 0.001] and [95.5 % vs. 26.3 %, OR = 58.8 (95 % CI, 19.86-174.2), P < 0.0001, respectively] (Table 3 and Figure 3a and Figure 3b) and the frequency of habitual alcohol drinker was significantly lower (18.2 % vs. 54.4 %, P < 0.001) in the $ALDH2^*2$ group than in the $ALDH2^*1^*/1$ group (Table 3). AFS was significantly associated with CSA [67.5 % vs. 39.8 %, OR=3.15 (95 % CI, 1.77-5.63), P < 0.001] and $ALDH2^*2$ [73.7 % vs. 4.5 %, OR=58.80 (95 % CI, 19.84-174.2), P < 0.0001] (Figure 4a and 4b). AFS had 95.5 % (84/88) sensitivity and 73.7 % (84/114) specificity for $ALDH2^*2$ and thus may be useful for detecting $ALDH2^*2$ (Table 3). $ALDH2^*2$ had a 55.4 % (62/112) sensitivity and 71.1 % (64/90) specificity for CSA (Table 2) and 73.7 % (84/114) sensitivity and 95.5 % (84/88) specificity for AFS, respectively.

In 138 subjects without significant organic coronary stenosis, AFS had 93.0 % (53/57) sensitivity and 72.8 % (59/81) specificity for $ALDH2^*$. $ALDH2^*2$ had a 52.1 % (38/73) sensitivity and 70.8 % (46/65) specificity for CSA and 70.7 % (53/75) sensitivity and 93.7 % (59/63) specificity for AFS. There was no significant difference of organic stenosis in the coronary arteries in the both groups (28.9 % in the wild $ALDH2$ group and 35.2 % in the variant
The multivariable logistic regression analyses for the predictors of CSA were performed including age (> 65 yrs.), gender, *ALDH2*2 genotype, alcohol flushing syndrome, tobacco smoking, plasma levels of uric acid (> 6.0 mg/dL) and HDL cholesterol (> 60 mg/dL) as an independent variable on the basis of theoretical and clinical grounds (age, gender and smoking),1 strength of associations (tobacco smoking, gender, AFS, *ALDH2*2, plasma levels of uric acid and HDL cholesterol) and collinearity (AFS). Because there was a highly significant relationship between *ALDH2*2 genotype and AFS [OR = 58.8 (95 % CI, 19.8-174.2), P < 0.0001], the final models included *ALDH2*2 genotype in conjunction with age, male gender, and smoking, HDL-cholesterol and uric acid and excluded AFS as an independent variable. The analyses revealed that *ALDH2*2 genotype and smoking were significant predictors of CSA (OR = 3.61; 95 % CI, 1.87-6.94; P < 0.001 and OR = 2.32, 95 % CI, 1.12-4.79, respectively) (Table 4).

Discussion

CSA is a common disease among East Asians but is rare in the Western countries.1-4 East Asians show rapid and intense alcohol flushing after drinking alcohol in the amount that has no effect on Caucasians in up to about half of the population.5-7 This is due to the presence of the variant of *ALDH2* genotype with a substitution of glutamate to lysine at position 504 (Glu504Lys) or *ALDH2*2 which is present only in East Asians but is virtually absent in other populations of the world.5-7 *ALDH2*2 exerts a dominant negative effect over wild-type homozygote *ALDH2*1*1 and heterozygote *ALDH2*1*2 show a severely reduced and homozygotes *ALDH2*2*2 negligible ALDH2 activity.17,18 The carriers of *ALDH2*2 thus manifest the characteristic AFS caused by accumulation of ethanol-derived acetaldehyde.5-7 We have shown that CSA is associated with
AFS.\textsuperscript{9} We and others reported also that CSA was induced by alcohol drinking, particularly in those with AFS.\textsuperscript{10,11}

The present study demonstrates for the first time that the frequencies of variant $ALDH2^*2$ genotype and AFS are significantly higher in the CSA group than non-CSA group. Conversely, the frequency of CSA and AFS were significantly higher in the variant $ALDH2^*2$ group than the wild $ALDH2^*1^*1$ group. The multiple logistic regression analyses revealed that $ALDH2^*2$ genotype and AFS were the most significant risk factors for CSA after adjusting for age, gender, tobacco smoking and other coronary risk factors. The present study thus reveals that $ALDH2^*2$ specifically present among East Asians is significantly associated with CSA and may thereby explain at least partially why CSA is common among East Asians. However, the fact that 29 \% of the carriers of $ALDH2^*2$ did not exhibit, while 44 \% of the non-carriers of $ALDH2^*2$ exhibited CSA (Figure 3a) implies that other factors than $ALDH2^*2$ are involved in the pathogenesis of CSA. Indeed, the previous studies reported that the environmental\textsuperscript{1,2,19,20} and genetic factors\textsuperscript{21-24} other than $ALDH2^*2$ were also associated with CSA. These and other unknown factors also may thus affect ALDH2 activity. It is therefore likely that $ALDH2^*2$ itself is a significant but may not be a major player in the pathogenesis of CSA, affecting CSA only through deficient ALDH2 activity. On the other hands, $ALDH2^*2$ had a highly significant association with AFS (OR = 58.8, $P < 0.0001$) in agreement with the results of previous studies\textsuperscript{6-7} and may plays a major role in the production of AFS through deficient ALDH2 activity and hence elevation of acetaldehyde on alcohol intake, although other factors may also be involved (Figure 3b).\textsuperscript{6,7,25-27} AFS may therefore be useful as a convenient marker for $ALDH2^*2$ in the absence of genotyping for $ALDH2^*2$.

There is the possibility that other genotypes related to AFS may also be related to CSA.
and possibly ALDH2 activity. However, there were no differences in the frequencies of *ADH1B* genotypes which convert alcohol into acetaldehyde between the CSA and control groups and a great majority (more than 90%) of the study subjects were the carriers of active variant *ADH1B*2 in both groups in the present study. This is in agreement with the previous studies showing that the prevalence of *ADH1B*2 is high among East Asians but is rare among other populations of the world.6,7,25-27 The enhanced enzyme activity of ADH1B*2 would therefore interact synergistically with the deficient activity of ALDH2*2 enzyme and enhance the accumulation of acetaldehyde leading to alcohol flushing syndrome. Recent studies showed that there is an additional effect of *ADH1B*2 on level of blood acetaldehyde in response to alcohol, but only among individuals with the *ALDH2*1/*2 genotype and therefore *ALDH2*2, rather than *ADH1B*2*2 is a causal variant allele for the accumulation of blood acetaldehyde and the resultant facial flushing during low alcohol consumption.7,26,27 Other genetic and environmental factors also may affect ALDH2 activity and hence blood acetaldehydes.20-23,28-32

**Clinical implications**

Oxidative degradation of lipid membrane (lipid peroxidation) generates numerous reactive aldehydes and causes oxidative damage.28,29 ALDH2 activity eliminates not only acetaldehyde but also other toxic aldehydes including 4-hydroxy-2 nonenal (4-HNE), and malondialdehyde (MDA) from lipid peroxidation or acrolein in tobacco smoke, thereby protecting tissues and cells from oxidative damage.28-31 Conversely, ALDH2 activity is suppressed by reactive oxygen species (ROS) and/or aldehydes.28-32 It is thus likely that the reactive aldehydes will be increased in the presence of deficient ALDH2 activity or *ALDH2*2.28-32 The present study therefore identified the deficient ALDH2 activity and hence reactive aldehydes and ROS as risk factors for CSA. Indeed, CSA patients have increased reactive oxygen species1,19,20,22,23 and are liable to
acute myocardial infarction (MI).\textsuperscript{1,33,34} \textit{ALDH2*2} genotypes are reported to be an important risk factor for MI in East Asians including Japanese, Koreans and Chinese.\textsuperscript{35-39} Takeuchi and coworkers recently identified the genetic locus of \textit{ALDH2*2} (rs671) as the strongest predictor for MI on a genome-wide association study (GWAS) in Japanese.\textsuperscript{40} \textit{ALDH2} also plays an essential role in the bioactivation of nitroglycerin widely used for the treatment of ischemic heart disease.\textsuperscript{18,32,41} However, continued administration of nitroglycerin leads to the tolerance\textsuperscript{18,32,41,42} or even cardiac events\textsuperscript{43} through inactivation of \textit{ALDH2} enzyme and an increased ROS.\textsuperscript{18,32,41,42} Accordingly, the carriers of \textit{ALDH2*2} genotypes are less responsive to nitroglycerin and are also susceptible to nitroglycerin tolerance and ROS.\textsuperscript{18,32,41,42}

It is estimated that more than 500 million (or 8\%) of the world population are the carriers of \textit{ALDH2*2} genotype.\textsuperscript{31} However, a considerable number of the subjects with \textit{ALDH2*2} genotype or even those with CSA are habitual alcohol drinkers\textsuperscript{6} as shown also in this study. Calcium-channel blockers are the mainstay in the treatment of CSA at present.\textsuperscript{1,2} However, there are considerable numbers of patients with CSA whose attacks cannot be controlled even by high doses of calcium-channel blockers or the combination with other drugs.\textsuperscript{1,2,33,34,43-45} The present study identified deficient \textit{ALDH2} enzyme activity and hence increased reactive aldehydes as risk factors to be targeted and intervened for the treatment and prevention of CSA. Recently, Chen and colleagues showed that a novel small molecule activator of \textit{ALDH2}, Alda-1, enhanced the activity of \textit{ALDH2} and effectively restored the activity of variant \textit{ALDH2*2} to wild-type levels in animal models.\textsuperscript{30,31}

\textbf{Limitations}

The number of study patients was small because the study was invasive and required a high degree of expertise. The study subjects were a select population who underwent coronary
angiography on suspicion of CSA, and this may have altered the relationships to the \textit{ALDH2} variant. Thus, the association found in this study need confirmation in other patient groups. However, the presence or absence of CSA was strictly determined by angiographic documentation. The study subjects were limited to the Japanese suspected of CSA because of the genetic association study\textsuperscript{16} and thus the results of this study may not be necessarily applicable to other populations. The frequency of AFS was assessed on questionnaire survey and recall and subjective biases may have influenced the results.

**Conclusions**

East Asian variant of \textit{ALDH2}*2 genotype and AFS were associated with CSA in the Japanese. This study thus identified deficient ALDH2 activity and hence increased reactive aldehydes as risk factors to be targeted and intervened for the treatment and prevention of CSA. AFS is a highly sensitive but suboptimal specific clinical marker of \textit{ALDH2}*2 genotype.

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**Conflict of Interest Disclosures:** None.

**References:**


Table 1. Comparison of the baseline characteristics between CSA and Control groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CSA (n = 112)</th>
<th>Control (n = 90)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66.3 ± 10.9</td>
<td>66.2 ± 12.0</td>
<td>0.927</td>
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<tr>
<td>Gender (Male), n (%)</td>
<td>79 (70.5)</td>
<td>40 (44.4)</td>
<td>&lt;0.001</td>
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<td>BMI, kg/m²</td>
<td>24.0 ± 3.1</td>
<td>24.2 ± 3.3</td>
<td>0.649</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>131.0 ± 19.9</td>
<td>134.3 ± 24.9</td>
<td>0.291</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>74.8 ± 13.4</td>
<td>76.7 ± 18.4</td>
<td>0.324</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>0.80 (0.25, 2.31)</td>
<td>0.49 (0.24, 1.09)</td>
<td>0.126</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.8 (5.1, 6.9)</td>
<td>5.6 (5.2, 6.4)</td>
<td>0.494</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.90 (4.09, 5.62)</td>
<td>5.18 (4.39, 5.85)</td>
<td>0.105</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.28 (0.99, 1.81)</td>
<td>1.22 (0.96, 1.92)</td>
<td>0.656</td>
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<tr>
<td>HDL-C, mmol/L</td>
<td>1.34 (1.14, 1.66)</td>
<td>1.56 (1.32, 1.86)</td>
<td>&lt;0.001</td>
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<tr>
<td>LDL-C, mmol/L</td>
<td>2.81 (2.12, 3.30)</td>
<td>2.81 (2.25, 3.46)</td>
<td>0.497</td>
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<tr>
<td>Uric acid, µmol/L</td>
<td>339 (238, 393)</td>
<td>292 (256, 357)</td>
<td>0.007</td>
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<tr>
<td>e-GFR, mL/min./1.73m²</td>
<td>69.7 ± 16.8</td>
<td>68.0 ± 18.0</td>
<td>0.521</td>
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<td>Leukocyte, /µL</td>
<td>6300 (4900,7600)</td>
<td>5900 (5000,7100)</td>
<td>0.249</td>
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<tr>
<td>Hemoglobin, g/dl</td>
<td>13.9 ± 1.7</td>
<td>13.4 ± 1.6</td>
<td>0.029</td>
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<tr>
<td>Platelets, ×10⁹/µL</td>
<td>21.6 ± 7.2</td>
<td>21.1 ± 5.2</td>
<td>0.528</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>71 (63.4)</td>
<td>30 (33.3)</td>
<td>&lt;0.001</td>
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<td>Alcohol habit, n (%)</td>
<td>43 (38.4)</td>
<td>35 (38.9)</td>
<td>0.943</td>
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<td>Female, n (%)</td>
<td>39 (49.4)</td>
<td>29 (72.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>AFS, n (%)</td>
<td>77 (68.8)</td>
<td>37 (41.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Organic stenosis, n (%)</td>
<td>39 (34.8)</td>
<td>25 (27.8)</td>
<td>0.285</td>
</tr>
<tr>
<td>0 vessel disease, n (%)</td>
<td>73 (65.2)</td>
<td>65 (72.2)</td>
<td>0.285</td>
</tr>
<tr>
<td>1 vessel disease, n (%)</td>
<td>23 (20.5)</td>
<td>18 (20.0)</td>
<td>0.743</td>
</tr>
<tr>
<td>2 vessel disease, n (%)</td>
<td>16 (14.3)</td>
<td>7 (7.8)</td>
<td>0.148</td>
</tr>
</tbody>
</table>

BMI indicates Body mass index; BP, blood pressure; CSA, coronary spastic angina; e-GFR, estimated glomerular filtration rate; AFS, alcohol flushing syndrome; HDL-C, high-density lipoprotein-cholesterol; Hs-CRP, high-sensitivity-C reactive protein; LDL-C, low-density lipoprotein-cholesterol; and synd., syndrome.
Table 2. Frequency distribution of the genotypes for ADH1B and ALDH2.

<table>
<thead>
<tr>
<th></th>
<th>CSA (n = 112)</th>
<th>Control (n = 90)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADH1B gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADH1B*1/*1, n (%)</td>
<td>5 (4.5)</td>
<td>5 (5.6)</td>
<td>0.722</td>
</tr>
<tr>
<td>ADH1B*1/*2, n (%)</td>
<td>42 (37.5)</td>
<td>37 (41.1)</td>
<td>0.601</td>
</tr>
<tr>
<td>ADH1B*2/*2, n (%)</td>
<td>65 (58.0)</td>
<td>48 (53.3)</td>
<td>0.503</td>
</tr>
<tr>
<td><strong>ALDH2 gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALDH2*1/*1, n (%)</td>
<td>50 (44.6)</td>
<td>64 (71.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALDH2*1/*2, n (%)</td>
<td>52 (46.4)</td>
<td>24 (26.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>ALDH2*2/*2, n (%)</td>
<td>10 (8.9)</td>
<td>2 (2.2)</td>
<td>0.088</td>
</tr>
</tbody>
</table>

ADH1B indicates alcohol dehydrogenase 1 beta; ALDH2, aldehyde dehydrogenase 2; *1/*1, wild homozygote; *1/*2, variant heterozygote; and *2/*2, variant homozygote.
Table 3. Comparison of the clinical characteristics between Variant and Wild ALDH2 genotypes.

<table>
<thead>
<tr>
<th>Variables</th>
<th>ALDH2*2 (Variant) (n = 88)</th>
<th>ALDH2<em>1</em>1 (Wild) (n = 114)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>65.8 ± 10.5</td>
<td>66.6 ± 12.1</td>
<td>0.611</td>
</tr>
<tr>
<td>Gender (Male), n (%)</td>
<td>49 (55.7)</td>
<td>70 (61.4)</td>
<td>0.412</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4 ± 3.5</td>
<td>23.9 ± 3.0</td>
<td>0.298</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>132.8 ± 21.9</td>
<td>132.3 ± 22.7</td>
<td>0.880</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>74.1 ± 15.1</td>
<td>76.5 ± 16.4</td>
<td>0.273</td>
</tr>
<tr>
<td>Hs-CRP, mg/L</td>
<td>0.83 (0.33, 2.26)</td>
<td>0.51 (0.22, 1.55)</td>
<td>0.063</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.6 (5.0, 6.4)</td>
<td>5.8 (5.2, 6.8)</td>
<td>0.320</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.94 (4.25, 5.72)</td>
<td>4.98 (4.23, 5.76)</td>
<td>0.558</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.24 (1.04, 1.75)</td>
<td>1.29 (0.92, 1.95)</td>
<td>0.895</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.40 (1.15, 1.73)</td>
<td>1.50 (1.27, 1.76)</td>
<td>0.151</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.84 (2.22, 3.45)</td>
<td>2.72 (1.99, 3.41)</td>
<td>0.505</td>
</tr>
<tr>
<td>Uric acid, µmol/L</td>
<td>297 (255, 368)</td>
<td>338 (237, 386)</td>
<td>0.160</td>
</tr>
<tr>
<td>e-GFR, mL/min./1.73m²</td>
<td>68.2 ± 16.9</td>
<td>69.6 ± 17.7</td>
<td>0.613</td>
</tr>
<tr>
<td>Leukocyte, /µL</td>
<td>6200 (5000, 7600)</td>
<td>5850 (4800, 7100)</td>
<td>0.164</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>13.7 ± 1.7</td>
<td>13.7 ± 1.7</td>
<td>0.937</td>
</tr>
<tr>
<td>Platelets, ×10⁴/µL</td>
<td>22.2 ± 6.1</td>
<td>20.8 ± 6.6</td>
<td>0.138</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>48 (54.5)</td>
<td>53 (46.5)</td>
<td>0.256</td>
</tr>
<tr>
<td>ADH1B*1/*1, n (%)</td>
<td>6 (6.8)</td>
<td>4 (3.5)</td>
<td>0.337</td>
</tr>
<tr>
<td>ADH1B*1/*2, n (%)</td>
<td>38 (43.2)</td>
<td>41 (36.0)</td>
<td>0.297</td>
</tr>
<tr>
<td>ADH1B*2/*2, n (%)</td>
<td>44 (50.0)</td>
<td>69 (60.5)</td>
<td>0.135</td>
</tr>
<tr>
<td>Alcohol habit, n (%)</td>
<td>16 (18.2)</td>
<td>62 (54.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>14 (28.6)</td>
<td>54 (77.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>2 (5.1)</td>
<td>8 (18.2)</td>
<td>0.094</td>
</tr>
<tr>
<td>AFS, n (%)</td>
<td>84 (95.5)</td>
<td>30 (26.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CSA, n (%)</td>
<td>62 (70.5)</td>
<td>50 (43.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Organic stenosis, n (%)</td>
<td>31 (35.2)</td>
<td>33 (28.9)</td>
<td>0.341</td>
</tr>
<tr>
<td>0 vessel disease, n (%)</td>
<td>57 (64.8)</td>
<td>81 (71.1)</td>
<td>0.341</td>
</tr>
<tr>
<td>1 vessel disease, n (%)</td>
<td>20 (22.7)</td>
<td>21 (18.4)</td>
<td>0.450</td>
</tr>
<tr>
<td>2 vessel disease, n (%)</td>
<td>11 (12.5)</td>
<td>12 (10.5)</td>
<td>0.662</td>
</tr>
</tbody>
</table>

ALDH2 indicates alcohol dehydrogenase 2; ALDH2, aldehyde dehydrogenase 2; ALDH2*2, variant ALDH2 genotype; ALDH2*1*1, wild homozygous ALDH2 genotype; BMI, body mass index; BP, blood pressure; CSA, coronary spastic angina; e-GFR, estimated glomerular filtration rate; AFS, alcohol flushing syndrome; HDL-C, high-density lipoprotein-cholesterol; Hs-CRP, high-sensitivity-C reactive protein; LDL-C, low-density lipoprotein-cholesterol; *1/*1, wild homozygote; *1/*2, variant heterozygote; and *2/*2, variant homozygote.
Table 4. The multivariable logistic regression analysis for CSA.

|                | OR  | Std. Err | z    | p>|z| | 95% CI     |
|----------------|-----|----------|------|-----|-----------|
| Age            | 1.342 | 0.435 | 0.91 | 0.363 | 0.712 - 2.532 |
| Gender (Male)  | 1.845 | 0.744 | 1.52 | 0.129 | 0.837 - 4.066 |
| ALDH2 *2       | 3.607 | 1.205 | 3.84 | 0.000 | 1.874 - 6.943 |
| Smoking        | 2.320 | 0.859 | 2.27 | 0.023 | 1.123 - 4.794 |
| HDL-C          | 0.860 | 0.283 | -0.46 | 0.648 | 0.452 - 1.639 |
| Uric Acid      | 1.271 | 0.170 | 1.79 | 0.074 | 0.977 - 1.652 |

ALDH2*2 indicates aldehyde dehydrogenase 2 variant genotype; CI, confidence interval; CSA, coronary spastic angina; HDL-C, high-density lipoprotein-cholesterol; OR, odds ratio; and Std. Err, Standard Error.

Figure Legends:

Figure 1. Coronary angiograms and ECG induced by intracoronary ACh infusion. a) a diffuse severe vasoconstriction of the LCA with ST elevation on ECG appeared ACh dose-dependently. Spasm with ST elevation also appeared at the RCA and disappeared after GTN. b) A diffuse severe vasoconstriction with ST depression on ECG of the LCA was induced after ACh and the spasm site changed within a minute at the LCA. The diffuse severe vasoconstriction with ST depression was also induced at the RCA. The arrow head indicates the site of disappearance of flow. ACh indicates acetylcholine; GTN, nitroglycerin; LCA, left coronary artery; and RCA, right coronary artery.

Figure 2. Comparison of frequency of coronary spastic angina (CSA) by genotype of ADH1B and ALDH2 group. (a) There was no difference in the frequency of CSA by ADH1B genotype. (b) On the other hands, the frequency of CSA was significantly higher in the variant ALDH2*2 genotypes as compared to wild genotype ALDH2*1/*1. ADH1B indicates alcohol dehydrogenase subunit 1 beta; ALDH2, aldehyde dehydrogenase 2; and CSA, coronary spastic angina.
Figure 3. Comparison of frequency of coronary spastic angina (CSA) and alcohol flushing syndrome by ALDH2 genotype group. The frequencies of CSA (a) and alcohol flushing response (b) were both significantly higher in the variant genotype ALDH2*2 group as compared to the wild genotype ALDH2*1/*1 group. ALDH2 indicates aldehyde dehydrogenase 2; and CSA, coronary spastic angina.

Figure 4. Comparison of frequency of coronary spastic angina (CSA) and ALDH2 genotype by alcohol flushing syndrome group. The frequencies of CSA (a) and variant ALDH2 genotypes (b) were significantly higher in the flushing group as compared to the control group. ALDH2 indicates aldehyde dehydrogenase 2; and CSA, coronary spastic angina.
Figure 1
Figure 2

Coronary Spastic Angina

- **ADH1B *1*1** (n=10)
  - 50.0%

- **ADH1B *2*1** (n=79)
  - 53.2%

- **ADH1B *2*2** (n=113)
  - 57.5%

- **ALDH2 *1*1** (n=114)
  - 43.9%

- **ALDH2 *2*1** (n=76)
  - 68.4%

- **ALDH2 *2*2** (n=12)
  - 83.3%

*P* = 0.785

*P* < 0.001
Coronary Spastic Angina

ALDH2 *1*1 (n=114)
43.9%
ALDH2 *2 (n=88)
70.5%

Alcohol Flushing

ALDH2 *1*1 (n=114)
26.3%
ALDH2 *2 (n=88)
95.5%

Figure 3
Figure 4
East Asian Variant of Aldehyde Dehydrogenase 2 (ALDH2*2) is Associated with Coronary Spastic Angina: Possible Roles of Reactive Aldehydes and Implications of Alcohol Flushing Syndrome

Yuji Mizuno, Eisaku Harada, Sumio Morita, Kenji Kinoshita, Mariko Hayashida, Makoto Shono, Yoshinobu Morikawa, Toyoaki Murohara, Masafumi Nakayama, Michihiro Yoshimura and Hirofumi Yasue

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유전적 요인이 이형협심증의 발생 위험을 높인다

강 현 재 교수 서울대학교병원 순환기내과

초록

배경
이형협심증은 일본인을 포함한 동아시아인에게 흔한 질환이다. 알코올 홍조 증후군(alcohol flushing syndrome)은 aldehyde dehydrogenase 2의 기능결핍 유전자 변이(ALDH2*2 유전자형)와 연관성이 있으며, 동아시아인에서 흔하다. 연구자들은 이형협심증과 ALDH2*2 유전자형의 관계를 일본인에서 평가하였다.

방법 및 결과
본 연구는 이형협심증의 의심 하에 관상동맥 내 acetylcholine 주입과 관상동맥 조영술을 시행한 202명(남성 119명, 여성 83명; 평균 연령 66.2±11.4세)에 대하여 시행되었다. 대상자들은 이형협심증군(112명)과 대조군(90명)으로 나누어서 분석하였다. ALDH2 유전자형 분석은 건조한 전월에 대하여 TaqMan 중합효소 연쇄반응(polymerase chain reaction) 시스템으로 시행하였다. 임상 및 실험실 검사 결과는 일반적인 방법으로 측정하였다. 이형협심증군에는 대조군보다 남성, ALDH2*2 유전자, 알코올 홍조 증후군, 흡연의 빈도, 그리고 혈장 내 요산의 농도가 높았고(각각 P<0.001, P<0.001, P<0.001, P<0.001, 그리고 P=0.007), 혈장의 고밀도 지단백 콜레스테롤의 농도가 낮았다(P<0.001). 다변수 로지스틱 회귀분석을 시행한 결과. ALDH2*2 유전자형과 흡연은 이형협심증과 유의하게 연관성이 있었다(각각 P<0.001, P=0.024).

결론
동아시아인에서 흔히 관찰되는 ALDH2*2 유전자형과 그와 연관된 ALDH2의 기능결핍은 일본인에서 이형협심증과 연관성이 있다. 본 연구는 이형협심증에서 aldehyde를 목표로 하는 치료법에 대한 연구의 필요성을 보여주고 있다.