# Role of aldehyde dehydrogenase 2 Glu504lys polymorphism in acute coronary syndrome

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## Abstract

This study aimed to investigate the association of the aldehyde dehydrogenase 2 (ALDH2) Glu504Lys polymorphism, which exists in 30–50% of East Asians, and risk of acute coronary syndrome (ACS). We enrolled 1092 unrelated Han Chinese, including 546 with ACS and 546 age- and sex-matched controls. Subjects with ALDH2 mutant genotypes showed significantly higher ACS than did controls (46.7% *versus* 31.9%, $P < 0.001$). Logistic regression analysis revealed the ALDH2 mutant independently associated with ACS (odds ratio [OR] 1.95, 95% confidence interval [CI]: 1.31–2.92, $P = 0.001$), but the association was weaker on adjusting for alcohol consumption (OR 1.82, 95% CI: 1.23–2.70, $P = 0.003$). Similar results were found in a subgroup analysis of patients with primary ST-segment elevation myocardial infarction (STEMI). The ALDH2 mutant was significantly associated with level of high-sensitivity C-reactive protein (hs-CRP) in patients with ACS ($P = 0.002$) and in controls ($P = 0.009$) and number of circulating endothelial progenitor cells (EPCs) ($P = 0.032$); furthermore, inclusion of hs-CRP level and EPCs number as independent variables in regression analysis reduced the importance of ALDH2 polymorphism in ACS or primary STEMI. However, ALDH2 polymorphism was not associated with number of coronary arteries with significant stenosis, Gensini score or flow-mediated dilation of the brachial artery. Our results suggest that ALDH2 mutation is a genetic risk marker for ACS, which is explained in part by alcohol consumption, inflammation and number of circulating EPCs.

**Keywords:** acute coronary syndrome • aldehyde dehydrogenase 2 • Glu504Lys polymorphism • inflammation • endothelial progenitor cells

## Introduction

An association of alcohol consumption and coronary artery disease (CAD) has been suggested [1, 2]. Gene polymorphisms of key enzymes in alcohol metabolism, including alcohol dehydrogenase 3 (ADH3) and aldehyde dehydrogenase 2 (ALDH2), could be implicated in the association of alcohol consumption and CAD [3–6]. The ADH3 gene polymorphism, common in Caucasian populations, was found to be involved in the association of alcohol consumption and myocardial infarction (MI) [6]. The Glu504Lys single nucleotide polymorphism in the ALDH2 gene exists mainly in East Asians. Between 30% and 50% of East Asians (or 6% the world's population) carry the mutant allele of ALDH2 [7–9]. Recently, cross-sectional studies in East Asia, including our pilot study, demonstrated a higher prevalence of the ALDH2 Glu504Lys mutant in patients with MI than in control populations [3–5]. An *in vitro* study showed that ALDH2 derived from the wild type and mutant types had different activities and thus notably different effects on the extent of myocardial necrosis in acute MI [7]. These findings suggest that the ALDH2 Glu504Lys polymorphism may play a functional role in CAD. However, the association of ALDH2 Glu504Lys polymorphism and acute coronary syndrome (ACS), and the potential mechanisms involved have not been examined in Han Chinese.

The ALDH2 enzyme is mainly located in mitochondria and is encoded by the ALDH2 gene on chromosome 12. The ALDH2 gene is composed of 13 exons. Exon 12 contains a G-to-A missense

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mutation, whereby glutamate at position 504 is replaced by lysine (Glu504Lys); hence, two ALDH2 alleles exist (Glu504 and Lys504, also named ALDH2*1 and ALDH2*2) with three genotypes, namely *1/*1 (wild-type homozygote), *1/*2 (heterozygote) and *2/*2 (mutant homozygote). Studies suggest that ALDH2 is the key enzyme for both alcohol and nitroglycerin metabolism [10, 11]. The mutant ALDH2*2 is involved in the transformation from nitroglycerin to nitric oxide, which plays a key role in nitroglycerin tolerance among Asian patients with CAD [10]. A study in a canine model also showed that different ALDH2 genotypes could influence coronary artery dilatation triggered by nitroglycerin [11]. These studies suggest that ALDH2 could influence coronary spasm-associated ACS. In addition, level of high-sensitivity C-reactive protein (hs-CRP), a classical inflammatory biomarker and number of circulating endothelial progenitor cells (EPCs) are associated with vulnerable plaque which has been recognized as a fundamental mechanism of ACS [12–14].

In this study, we first investigated the association of the ALDH2 polymorphism and ACS in Han Chinese. We then studied whether the association is related to alcohol consumption, vasodilatation, level of hs-CRP and number of circulating EPCs, which could be the mediating mechanism for the association.

# Materials and methods

## Study protocol

Consecutive Han Chinese patients with ACS who underwent coronary angiography in our hospital were included from September 2007 to December 2008. The inclusion criteria were (1) clinical manifestations or electrocardiographic changes meeting the diagnostic criteria for unstable angina/non-ST-segment elevation myocardial infarction (NSTEMI) and STEMI defined by the American Heart Association/American College of Cardiology guidelines; (2) coronary angiography demonstrating at least one coronary lesion with luminal stenosis ≥50% and (3) no hereditary relationship between patients. Patients who had undergone intravenous thrombolysis, percutaneous coronary intervention or coronary artery bypass grafting before this coronary angiography were excluded. We also designed a subgroup analysis of patients with primary STEMI. Data for this subgroup were extracted from that for enrolled ACS patients; patients with a history of MI before the current event or electrocardiographic or echocardiographic evidence suggesting a history of MI were excluded from this subgroup. The control population was gender- and age (±1 year)-matched Han Chinese subjects without CAD recruited from the Health Examination Center of Qilu Hospital, Shandong University and patients with a negative coronary angiographic finding. Confirmation of most non-CAD control subjects was based on negative history of coronary artery diseases, absence of chest pain symptoms and normal electrocardiographic results; confirmation for some ($n = 62$) was based on normal coronary angiographic results. The controls also had no inflammatory diseases, autoimmune or malignant diseases, or other chronic diseases, except for hypertension (defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg or treatment, e.g. with antihypertensive drugs), diabetes and dyslipidemia; had no fever during the previous 2 weeks; and had not received long-term statin or hormone replacement therapy.

This study was approved by the Ethics Committee of Qilu Hospital, Shandong University in accordance with the guidelines of the 1975 Declaration of Helsinki. All subjects provided their informed consent to the study. During the first 24 hrs after admission, blood samples were collected from all patients.

## Alcohol consumption

The frequency of drinking (drinking days per week) and the average daily alcohol consumption were evaluated as previously described [6, 15]. Namely, the usual frequency of drinking and average amount of beer, wine or liquor during the participant's drinking lifetime was recorded by use of a semiquantitative questionnaire; 355 ml of beer, 118 ml of wine or 40 ml of liquor were defined as one drink and considered to contain the same amount of alcohol (approximately 14 g). The average daily alcohol consumption was calculated by the frequency of consumption and the amount of each beverage. No patients who frequently (more than once per week) drank hard liquor were enrolled.

## ALDH2 genotyping

The ALDH2 polymorphism was detected as we described previously [3]. In brief, DNA was extracted from 200 μl of venous blood; genomic DNA underwent PCR amplification; finally, PCR products were purified and directly sequenced by Invitrogen Corp. (Shanghai, China).

## Severity of CAD

All ACS patients underwent coronary angiography by the Judkins' method. For each major epicardial coronary artery, at least two views were observed. The degree of coronary stenosis was assessed by quantitative coronary angiography and luminal diameter stenosis ≥50% was defined as vessels with significant disease. The following two parameters were used to assess the severity of CAD: (1) the number of coronary arteries with significant stenosis and (2) Gensini score. The methods of evaluation have been described previously [3].

## Brachial artery vasodilatation

From September 2007 to January 2008, a subset of consecutive ACS patients ($n = 166$) underwent high-resolution ultrasonography (HP Sonos 7500, Philips Medical Systems, Andover, MA, USA) to detect flow-mediated dilation (FMD) and non-flow mediated dilation (NMD) of the brachial arteries. The ultrasonography was performed within 72 hr after coronary angiography, and vasodilative medications was stopped 18 hr before scanning. After patients rested for 30 min, ultrasonography was performed by a physician blinded to the study protocol using previously reported methods [16]. With the patient in a supine position, the diameters of the brachial arteries at rest (baseline) and in response to reactive hyperemia (FMD) and sublingual administration e.g. of nitroglycerin (NMD) were measured using a high-frequency probe (S12). The measurements were repeated for three times under each experimental condition and the values were averaged. The percentage increase of the brachial arterial diameters from the baseline value to the value measured at the peak of reactive hyperemia and to the value measured at the peak of nitroglycerin-induced vasodilation was taken as FMD and NMD, respectively.

## Inflammatory biomarkers in ACS and control subjects

In all ACS and control subjects, serum hs-CRP levels were measured by experts blinded to the study protocol using high-sensitivity latex-enhanced immunonephelometry with a commercial kit as described previously [17].

## Number of circulating EPCs in patients with primary STEMI

For all patients with primary STEMI, the number of peripheral circulating CD34 + KDR + EPCs was assessed by use of FACSCalibur cytometry (Becton Dickinson, NJ, USA). First, blood samples were incubated with FITC-labelled monoclonal mouse anti-human CD34 antibody (Jingmei Biotechnological Co, Wuhan, China) and PE-labelled KDR (R&D Systems, Minneapolis, MN, USA) for 20 min at room temperature away from the light or with the corresponding mouse anti-human isotype-matched control antibodies. Secondly, cells were further incubated with FACS lysing solution (Becton Dickinson) and washed with cold phosphate-buffered saline (PBS) three times. Finally, the samples and isotype controls were suspended with PBS and measured by FACSCalibur. At least 50,000 events were acquired in the lymphomonocytic gate; the number of circulating EPCs was expressed as proportion of CD34+ KDR+ cells to all lymphomonocytic cells.

# Statistical analysis

Quantitative data are expressed as mean $\pm$ S.D., or median and interquartile range; categorical data are presented as numbers and percentages. Student's t-test (the values of hs-CRP were compared after log transformation to achieve normal distribution) and Mann–Whitney nonparametric test (for triglycerides, alcohol consumption, Gensini score, EPCs) were used for analysis of quantitative data. chi-square test was used for analysis of categorical data. Numerical variables of abnormal distribution were first log transformed before they were included in regression analyses. Multivariate logistic and linear regression analyses were performed to examine the association of ALDH2 polymorphism with ACS, primary STEMI, severity of CAD, level of hs-CRP and number of circulating EPCs. We used multivariate regression analysis to control for age, gender, body mass index (BMI), lipid levels (total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol), history of smoking, hypertension, diabetes mellitus and family history of CAD. A two-tailed $P < 0.05$ was considered statistically significant. All data analysis involved use of SPSS v10.0 (SPSS Inc., Chicago, IL, USA).

# Results

## Demographic characteristics

A total of 1092 subjects were enrolled, including 546 patients with ACS and 546 non-CAD controls. Among ACS patients, 122 were diagnosed as having primary STEMI. The subgroup analysis of pri-

**Table 1** Demographic characteristics of patients with ACS and controls

| | ACS (*n* = 546) | Controls (*n* = 546) | *P*-value |
|---|---|---|---|
| Age (yrs) | 61.3 ± 11.2 | 61.3 ± 11.2 | NS |
| Male gender | 407 (74.5%) | 407 (74.5%) | NS |
| BMI (kg/m$^2$) | 25.6 ± 3.1 | 24.7 ± 3.4 | <0.001 |
| TC (mmol/l) | 4.69 ± 1.04 | 5.23 ± 0.96 | <0.001 |
| TG (mmol/l) | 1.52 (0.83) | 1.55 (0.99) | NS |
| LDL-C (mmol/l) | 2.99 ± 0.92 | 3.34 ± 0.78 | <0.001 |
| HDL-C (mmol/l) | 1.29 ± 0.34 | 1.37 ± 0.50 | <0.001 |
| Smoking | 299 (54.8%) | 289 (52.9%) | NS |
| Hypertension | 310 (56.8%) | 158 (28.9%) | 0.008 |
| Diabetes mellitus | 121 (22.2%) | 44 (8.1%) | <0.001 |
| Family history of CAD | 148 (27.1%) | 82 (15.0%) | <0.001 |
| Frequency of drinking | | | |
| ≥1 day per week | 193 (35.3%) | 152 (27.8%) | <0.001 |
| Alcohol consumption (g/day) | 2.9 (25.4) | 2.0 (18.7) | 0.032 |
| ALDH2*1/*2 + *2/*2 | 255 (46.7%) | 174 (31.9%) | <0.001 |

Data are mean $\pm$ S.D., median (interquartile range) or number of patients/controls (%). BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CAD: coronary artery disease.

mary STEMI was designed as a 1:2 control; 243 non-CAD control subjects were enrolled, including 122 who were from the initial matching control group and another 121 who were identified from the remaining 424 of all 546 gender- and age-matched controls (we could not identify a control who met the matching criteria for only 1 case). Demographic data for all subjects are in Tables 1 and 2.

## ALDH2 polymorphism and ACS or primary STEMI

In Han Chinese population, ALDH2*2 allele mostly exists in ALDH2*1/*2 heterozygote. Hence, in the present study, we pooled ALDH2 *1/*2 and *2/*2 into a single group as the mutant allele carrier. The ALDH2 genotypes for patients and controls were in Hardy-Weinberg equilibrium. More patients with ACS and primary STEMI showed ALDH2 mutant genotypes (*1/*2 and *2/*2) than did their controls (46.7% *versus* 31.9%, *P* < 0.001; 48.4% *versus* 32.9%, *P* = 0.006; respectively). In addition, patients with ACS with wild-type ALDH2 (*1/*1) showed significantly higher daily alcohol consumption and frequency of drinking than did those with mutant genotypes (*1/*2 + *2/*2), although the HDL-C level was markedly higher in the patients with wild type than those

**Table 2** Demographic characteristics of patients with primary STEMI and controls

| | Primary STEMI (n = 122) | Controls (n = 243) | P-value |
|---|---|---|---|
| Age (yrs) | 59.0 ± 9.5 | 59.4 ± 8.9 | NS |
| Male gender | 99 (81.1%) | 198 (81.5%) | NS |
| BMI (kg/m$^2$) | 25.3 ± 2.9 | 24.8 ± 3.0 | NS |
| TC (mmol/l) | 4.53 ± 1.12 | 5.17 ± 0.98 | <0.001 |
| TG (mmol/l) | 1.52 (0.76) | 1.49 (0.97) | NS |
| LDL-C (mmol/l) | 3.25 ± 0.59 | 3.31 ± 0.88 | NS |
| HDL-C (mmol/l) | 1.15 ± 0.28 | 1.40 ± 0.35 | <0.001 |
| Smoking | 80 (65.6%) | 145 (59.7%) | NS |
| Hypertension | 54 (44.3%) | 73 (30.0%) | 0.010 |
| Diabetes mellitus | 21 (17.2%) | 24 (9.9%) | NS |
| Family history of CAD | 35 (28.7%) | 41 (16.9%) | 0.013 |
| Frequency of drinking | | | |
| ≥1 day per week | 48 (39.3%) | 71 (29.2%) | NS |
| Alcohol consumption (g/day) | 3.2 (31.8) | 2.3 (16.0) | NS |
| ALDH2*1/*2 + *2/*2 | 59 (48.4%) | 80 (32.9%) | 0.006 |

Data are mean ± S.D., median (interquartile range) or number of patients/controls (%). BMI: body mass index; TC: total cholesterol; TG, triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CAD: coronary artery disease.

**Table 3** Characteristics of all ACS patients with ALDH2 genotypes

| | *1/*1 (n = 291) | *1/*2+*2/*2 (n = 255) | P-value |
|---|---|---|---|
| Age (yrs) | 61.5 ± 11.0 | 61.0 ± 11.2 | NS |
| Male gender | 207 (71.1%) | 200 (78.4%) | NS |
| BMI (kg/m$^2$) | 25.5 ± 3.1 | 25.7 ± 2.9 | NS |
| TC (mmol/l) | 4.71 ± 0.99 | 4.68 ± 1.10 | NS |
| TG (mmol/l) | 1.62 (0.78) | 1.52 (0.84) | NS |
| LDL-C (mmol/l) | 2.95 ± 0.93 | 3.03 ± 0.86 | NS |
| HDL-C (mmol/l) | 1.33 ± 0.32 | 1.24 ± 0.35 | 0.002 |
| Smoking | 158 (54.3%) | 141 (55.3%) | NS |
| Hypertension | 165 (56.7%) | 145 (56.9%) | NS |
| Diabetes mellitus | 57 (19.6%) | 64 (25.1%) | NS |
| Family history of CAD | 80 (27.5%) | 68 (26.7%) | NS |
| Frequency of drinking | | | |
| ≥1 days per week | 126 (43.3%) | 67 (26.3%) | <0.001 |
| Alcohol consumption (g/day) | 4.2 (36.4) | 0.8 (9.8) | <0.001 |
| Gensini score | 32 (70) | 44 (59) | NS |
| Number of significantly diseased coronary arteries | | | NS |
| 1 | 82 (28.2%) | 66 (25.9%) | |
| 2 | 99 (34.0%) | 89 (34.9%) | |
| 3 | 110 (37.8%) | 100 (39.2%) | |

Data are mean ± S.D., median (interquartile range) or number of patients (%). BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CAD: coronary artery disease.

with mutant genotypes (Table 3); similar results were found among the controls (data not shown). On binary logistic regression, ALDH2 mutant was an independent risk factor for ACS (odds ratio [OR] 1.95, 95% confidence interval [CI]: 1.31–2.92, P = 0.001) after controlling for age, BMI, lipid levels, history of smoking, hypertension, diabetes mellitus and family history of CAD. The association was weakened but still statistically significant when alcohol consumption was entered into the analytical model (OR 1.82, 95% CI: 1.23–2.70, P = 0.003); similar results were shown in the subgroup analysis of primary STEMI (OR 2.17, 95% CI: 1.30–3.62, P = 0.003; OR 1.95, 95% CI: 1.18–3.22, P = 0.009, after adjusting for alcohol consumption, respectively).

## ALDH2 polymorphism and severity of CAD

The 546 ACS patients did not differ by ALDH2 genotypes in Gensini score or number of significantly diseased coronary arteries (Table 3). Multivariate linear and logistic regression analyses demonstrated no association of ALDH2 polymorphism and Gensini score or number of significantly diseased coronary arteries after adjusting for age, gender, BMI, lipid levels, history of smoking, hypertension, diabetes mellitus and family history of CAD (all P > 0.05). Further analysis in the subset of patients with unstable angina with no history of MI (n = 326) also did not show any association (P > 0.05, respectively).

## ALDH2 polymorphism and artery vasodilatation

Table 4 shows the baseline characteristics of the 166 ACS patients with different ALDH2 genotypes who underwent ultrasonography. In these ACS patients, the diameter of the brachial artery at rest did not differ by genotype; FMD was also similar (4.9 ± 2.3% for

**Table 4** Baseline characteristics of 166 ACS patients with ALDH2 genotypes who underwent ultrasonography

|  | *1/*1 (*n* = 95) | *1/*2+*2/*2 (*n* = 71) | *P*-value |
|---|---|---|---|
| Age (yrs) | 57.9 ± 9.6 | 58.2 ± 11.8 | NS |
| Male gender | 67 (70.5%) | 52 (73.2%) | NS |
| BMI (kg/m$^2$) | 25.5 ± 2.7 | 25.9 ± 2.2 | NS |
| TC (mmol/l) | 4.70 ± 1.01 | 4.82 ± 1.26 | NS |
| TG (mmol/l) | 1.51 (0.84) | 1.54 (0.70) | NS |
| LDL-C (mmol/l) | 2.71 ± 0.69 | 2.91 ± 0.82 | NS |
| HDL-C (mmol/l) | 1.22 ± 0.33 | 1.15 ± 0.29 | NS |
| Smoking | 55 (57.9%) | 49 (69.0%) | NS |
| Hypertension | 56 (59.0%) | 41 (57.8%) | NS |
| Diabetes mellitus | 19 (20.0%) | 17 (23.9%) | NS |
| Family history of CAD | 21 (22.1%) | 18 (25.4%) | NS |

Data are mean ± S.D., median (interquartile range) or number of patients (%). BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CAD: coronary artery disease.



**Fig. 1** Serum level of high-sensitivity C-reactive protein (hs-CRP) in acute coronary syndrome (ACS) patients and controls by ALDH2 genotypes. Box plot analysis of hs-CRP level in Han Chinese with ACS and age- and gender-matched controls without coronary artery disease (CAD). The hs-CRP levels were significantly higher in the subjects with ALDH2 mutant genotypes (*1/*2 and *2/*2) than in those with wild-type genotype (*1/*1) among either ACS patients or non-CAD controls.
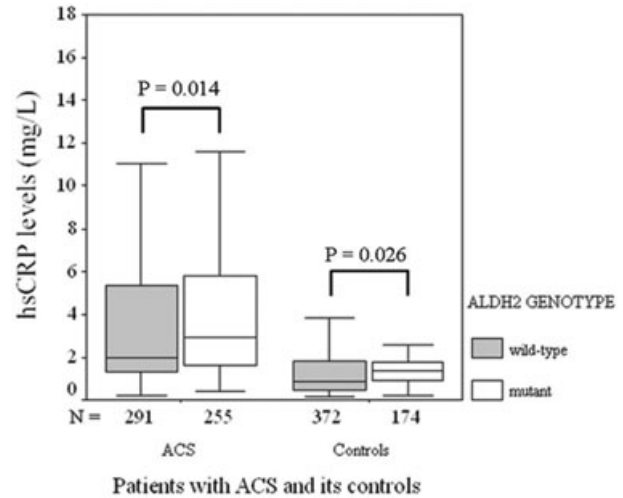
the mutant genotype *versus* 5.1 ± 2.5% for the wild type, *P* > 0.05). However, NMD in patients with mutant genotypes was significantly less in patients with than without the mutant ALDH2 genotype (3.9 ± 3.3% *versus* 10.5 ± 3.8%, *P* < 0.001).

### ALDH2 polymorphism and Hs-CRP level

Subjects with the ALDH2 mutant genotypes had higher hs-CRP levels than did those with the wild-type genotype, whether ACS patients or controls (Fig. 1). Moreover, multivariate linear regression revealed the ALDH2 mutation independently associated with high hs-CRP level in both ACS and control subjects (*P* = 0.002 and 0.009, respectively), with age, gender, BMI, lipid levels, history of smoking, hypertension and diabetes mellitus adjusted in the model. Furthermore, on analysing the association of ALDH2 polymorphism and ACS, inclusion of hs-CRP level as an independent variable weakened the importance of the ALDH2 mutant genotype in the regression model (OR 1.17, 95% CI: 1.01–1.37, *P* = 0.040).

### ALDH2 polymorphism and number of circulating EPCs

Table 5 shows the baseline characteristics of all primary STEMI patients with different ALDH2 genotypes. The primary STEMI patients with wild type tended to be older than those subjects

with mutant genotypes (*P* = 0.055), furthermore, the wild-type ALDH2 was associated with higher HDL-C level and lower TG level. In patients with primary STEMI, the number of circulating EPCs was significantly lower in subjects with ALDH2 mutant genotypes than in those with the wild-type genotype (Fig. 2). On multivariate linear regression, ALDH2 mutant genotype was an independent risk factor for a low number of circulating EPCs after controlling for age, gender, BMI, lipid levels, history of smoking, hypertension and diabetes mellitus (*P* = 0.032). However, binary logistic regression analysis revealed no association of ALDH2 genotypes and primary STEMI when hs-CRP level and number of circulating EPCs were included as independent variables as compared with traditional cardiovascular risk factors and alcohol consumption (*P* = 0.516).

## Discussion

The major finding of this study was that ALDH2 Glu504Lys polymorphism was significantly associated with ACS in Han Chinese and ALDH2 mutant genotypes (*1/*2 and *2/*2) were independent genetic risk factors for ACS. Another important finding was that the ALDH2 mutant was associated with level of hs-CRP and number of circulating EPCs and inclusion of hs-CRP level and number of circulating EPCs as independent variables in the regression model weakened the association of ALDH2 and ACS, suggesting

**Table 5** Baseline characteristics of all patients with primary STEMI by ALDH2 genotypes
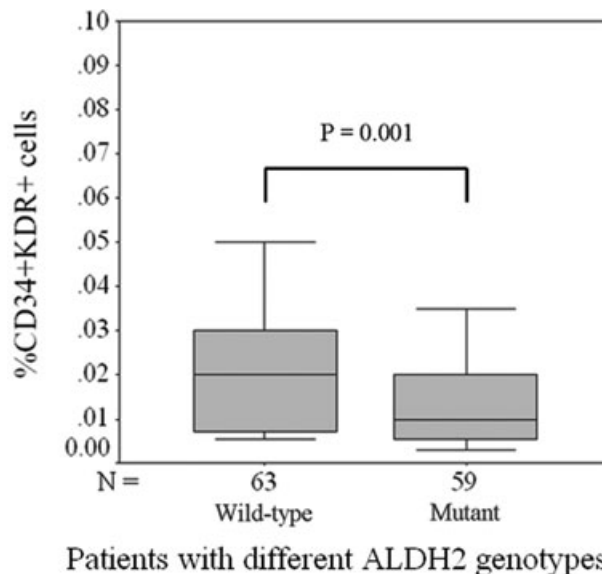
| | *1/*1 (n = 63) | *1/*2 + *2/*2 (n = 59) | P-Value |
|---|---|---|---|
| Age (yrs) | 60.6 ± 10.1 | 57.3 ± 8.6 | 0.055 |
| Male gender | 49 (77.8%) | 50 (84.8%) | NS |
| BMI (kg/m$^2$) | 25.2 ± 2.6 | 25.4 ± 3.1 | NS |
| TC (mmol/l) | 4.47 ± 1.12 | 4.60 ± 1.14 | NS |
| TG (mmol/l) | 1.48 (0.89) | 1.70 (1.03) | <0.001 |
| LDL-C (mmol/l) | 3.19 ± 0.63 | 3.31 ± 0.57 | NS |
| HDL-C (mmol/l) | 1.21 ± 0.31 | 1.09 ± 0.24 | 0.019 |
| Smoking | 40 (63.5%) | 40 (67.8%) | NS |
| Hypertension | 28 (44.4%) | 26 (44.1%) | NS |
| Diabetes mellitus | 7 (11.1%) | 14 (23.7%) | NS |
| Family history of CAD | 19 (30.2%) | 16 (27.1%) | NS |
| Frequency of drinking | | | |
| ≥1 days per week | 31 (49.2%) | 17 (28.8%) | 0.034 |
| Alcohol consumption (g/day) | 4.2 (25.4) | 0.6 (19.0) | <0.001 |

Data are mean ± S.D., median (interquartile range) or number of patients (%). BMI: body mass index; TC: total cholesterol; TG: triglycerides; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; CAD: coronary artery disease.



**Fig. 2** Levels of circulating CD34+/KDR+ endothelial progenitor cells (EPCs) in patients with STEMI and different ALDH2 genotypes. Box plot analysis of number of EPCs in the subgroup of ACS patients with STEMI. The number of circulating EPCs (expressed as proportion of CD34+KDR+ labelled cells to lymphomonocytes) was markedly lower in patients with ALDH2 mutant genotypes than in those with wild-type genotype.

that ALDH2 Gly504Lys polymorphism may affect plaque vulnerability and the occurrence of ACS. Although ALDH2 from mutant genes displays reduced activity for nitroglycerin [10], we found that subjects with different ALDH2 genotypes had similar FMD. Furthermore, ALDH2 mutation was not associated with severity of CAD as measured by the number of significantly diseased vessels and Gensini score.

Early studies demonstrated that the frequency of ALDH2*2 allele differs significantly among different races [8, 18]. The ALDH2*2 allele mainly exists in East Asians (30–50%), and approximately 6% of the world people carry ALDH2*2 allele. Therefore, exploring the association of ALDH2 polymorphism and ACS is valuable for the development of novel preventive and therapeutic strategies for ACS. The distribution of ALDH2*2 alleles also differs among different East Asian populations [5, 8, 18–20]. About 10% of healthy Japanese carry the ALDH2*2/*2 genotype as compared with only 4% of healthy Han Chinese people. In Han Chinese, the ALDH*2*2 allele mainly exists as the ALDH2*1/*2 heterozygote. Hence, in this study, we pooled the ALDH2*1/*2 and *2/*2 alleles into a single group for analysis as the mutant allele carrier.

Recently, investigators have begun to explore the association of ALDH2 polymorphism and CAD. A cross-sectional study

demonstrated the ALDH2*2/*2 polymorphism associated with MI in Japanese [5]. Our previous pilot study and a study in Korea showed similar results [3, 4]. However, the association of ALDH2 Glu504Lys polymorphism and the occurrence of ACS had never been studied in Han Chinese. In this study, we found that ALDH2 mutation was an independent genetic risk factor for ACS in Han Chinese, and a similar result was revealed in a subgroup analysis of patients with primary STEMI. Our findings further confirm the association of ALDH2 polymorphism with MI found in East Asians and suggest an important effect of this polymorphism on the progress of CAD [3–5].

ALDH2 is a key enzyme of alcohol metabolism. The activity of ALDH2 from *2 allele carriers is significantly lower than that of the *1/*1 homozygotes. Therefore, *2 allele carriers may have slower aldehyde metabolism after alcohol consumption, which may be associated with certain discomfort such as flushing, sweating, nausea, dysarthria, tachycardia and hypotension. Consequently, *2 allele carriers may voluntarily reduce their alcohol consumption to the extent of abstinence. Recently, a moderate consumption of alcohol was suggested to significantly reduce the incidence of cardiovascular events as compared with consumption of a little or a large amount of alcohol [2]. Therefore, the ALDH2 gene may influence the habit of alcohol consumption and thus the progression of cardiovascular disease. In this study, alcohol consumption was significantly lower in mutant than wild-type cases for both patients and controls, which is consistent with other studies in Chinese [8, 18, 21]. Moreover, with alcohol consumption included

as an independent variable in regression analysis, the association of the ALDH2 mutant genotype and ACS or primary STEMI was reduced. These results demonstrate that ALDH2 mutant genotypes increase the risk of ACS in part by significantly reducing patients' alcohol consumption. The association between ALDH2 mutant and HDL-C levels is in dispute. Our results showed that ALDH2 mutant was associated with lower HDL-C levels, which was consistent with a study in Japanese men [5]. However, another study in Japanese suggested that there was no association between ALDH2 mutant and HDL-C levels [22], and our study showed that the association of ALDH2 mutant and ACS was still significant after HDL-C was included for logistic analysis. These results suggest that the relationship between ALDH2 gene and HDL-C should be further investigated in studies with larger sample.

Previous studies showed that mitochondrial ALDH2 is an important nitrate reductase, which converts nitroglycerin to nitric oxide, and that the activity of ALDH2 from the mutant allele was significantly decreased as one type of nitrate reductase [10]. An experimental study in mongrel dogs demonstrated that coronary dilatation induced by nitroglycerin was significantly attenuated after treatment, *e.g.* with an ALDH2 inhibitor [11]. However, whether ALDH2 mutation influences coronary dilatation in human subjects remains unproved. In this study, we examined the association using high-resolution ultrasonography in a subset of ACS patients and found no difference in FMD among subjects with different genotypes, although the increase in brachial artery diameter after sublingual administration, *e.g.* of nitroglycerin was significantly lower in patients with than without the ALDH2 mutant allele. These results suggest a significant effect of ALDH2 mutant gene on NMD, which is consistent with previous findings [10, 11]. Previous studies found that impaired FMD, not NMD, was significantly associated with coronary artery spasm. Our results denied the possibility that the contribution of ALDH2 mutant genotypes to the incidence of ACS is due to triggering coronary artery spasm.

The finding that ALDH2 polymorphism is not associated with the severity of CAD as measured by the number of significantly diseased vessels and Gensini scores suggest that ALDH2 mutation may not participate in the development and progression of atherosclerosis. However, the hs-CRP level in subjects with mutant ALDH2 polymorphism was higher than in those with the wild-type polymorphism for both ACS patients and controls, and EPC numbers were lower in mutant allele carriers than in wild-type carriers with primary STEMI. Furthermore, inclusion of hs-CRP level and number of circulating EPCs as independent variables in regression analysis downplayed the importance of the ALDH2 polymorphism in ACS or primary STEMI. These findings suggest that ALDH2 could be involved in plaque vulnerability and rupture by inducing inflammation and reducing the number of circulating EPCs, leading to an acute coronary event [12–14]. This hypothesis is further supported by recent reports showing that ALDH2 activities with different ALDH2 genotypes had different impacts on oxidative stress and subsequent inflammation [7, 23–25]. However, the exact molecular mechanisms underlying the association of ALDH2 polymorphism and ACS remain to be elucidated.

In summary, this study demonstrates that ALDH2 mutation is an independent risk factor of ACS in Han Chinese. Vascular tone as measured by FMD and severity of atherosclerotic lesions cannot explain this relationship, but alcohol consumption, elevated hs-CRP level and decreased number of circulating EPCs could be responsible in part. Our findings suggest a novel target for the development of preventive and therapeutic strategies of ACS in East Asians, among whom 30–50% are mutant ALDH2 carriers.

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# Conflict of interest

The authors confirm that there were no conflicts of interest.

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