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Combined effects of current-smoking and the *aldehyde dehydrogenase 2*2* allele on the risk of myocardial infarction in Japanese patients

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HIGHLIGHTS

- Aldehyde dehydrogenase 2 (ALDH2) detoxifies toxic aldehydes in cigarette smoke.
- Current-smoking *ALDH2*2* allele carriers were at risk of myocardial infarction (MI).
- Current-smoking and *ALDH2*2* synergistically increased the peak creatine kinase.
- Current-smoking and *ALDH2*2* synergistically increased triglycerides in MI subjects.

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ABSTRACT

Aldehyde dehydrogenase 2 (ALDH2) detoxifies toxic aldehydes, e.g. acetaldehyde in cigarette smoke; however, the interactive effects between smoking status and the *ALDH2* genotype on coronary artery disease (CAD) have not been reported. We investigated the effects of smoking status and the *ALDH2* genotype, and assessed their interactive and combined effects on the risk of myocardial infarction (MI) or stable angina (SA), including 221 MI and 175 SA subjects and 473 age- and sex-matched controls without CAD. Current-smoking and the *ALDH2*2* allele additively increased the risk of MI (adjusted odds ratio 4.54, 95% confidence interval 2.25–9.15), although this combination was not associated with the risk of SA. This combination also increased the peak creatine kinase (CK) level synergistically in the acute MI (AMI) subjects. Moreover, current-smoking was found to be a significant risk factor for an increased peak CK level in the *ALDH2*2* allele carriers (B 2220.2 IU/L, $p = 0.008$), but not the non-carriers. Additionally, a synergistic effect of this combination on the triglycerides levels was also found in the AMI subjects. These preliminary findings suggest that the combination of current-smoking and the inactive *ALDH2*2* allele may increase the risk of MI additively and the infarct size synergistically.

1. Introduction

Both active and passive cigarette smoke exposure (CSE) increases the risk of coronary thrombosis and myocardial infarction (MI) (Ambrose and Barua, 2004; Barua and Ambrose, 2013). Although CSE is hazardous, only half of lifelong smokers die prematurely from smoking-related diseases (Barua and Ambrose, 2013). This discrepancy may be attributable to the presence of other risk factors and/or genetic predispositions.

Aldehyde dehydrogenase 2 (ALDH2) is the primary enzyme that detoxifies exogenous and endogenous toxic aldehydes in a range of organs and cell types and prevents free radical-mediated oxidative stress, which appears to play a central role in the pathogenesis of CSE-mediated atherothrombotic diseases (Ambrose and Barua, 2004; Barua and Ambrose, 2013; O'Brien et al., 2005; Stolle et al., 2010). The *ALDH2*1* and *ALDH2*2* alleles of rs671 encode the active

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and inactive subunits of ALDH2 respectively, the latter of which determines an individual's tolerance for alcohol consumption (O'Brien et al., 2005). A meta-analysis of genome-wide association studies (GWAS) identified the wild-type *ALDH2*1* allele to be a risk factor for elevated blood pressure (BP), and, conversely, the inactive *ALDH2*2* allele as a risk factor for coronary artery disease (CAD) in East Asians (Kato et al., 2011). Associations between the *ALDH2*2* variant and MI or acute coronary syndrome (ACS) have also been reported (Takagi et al., 2002; Takeuchi et al., 2012; Xu et al., 2011). These associations are believed to be largely mediated by low levels of high-density lipoprotein (HDL) cholesterol due to alcohol intolerance in variant carriers (Guo et al., 2010; Kato et al., 2011; O'Brien et al., 2005; Takagi et al., 2002; Xu et al., 2011).

ALDH2 detoxifies highly reactive aldehydes, such as acetaldehyde in cigarette smoke and 4-hydroxy-2-nonenal (4-HNE) generated by lipid peroxidation (Guo et al., 2010; Lee et al., 2006; O'Brien et al., 2005). Therefore, the inactive *ALDH2*2* variant has been identified to be a risk factor for smoking-related cancers but, surprisingly, not smoking-related CAD (Cui et al., 2009; Guo et al., 2010; Kato et al., 2011; Park et al., 2010; Takagi et al., 2002; Takeuchi et al., 2012; Xu et al., 2011). We herein report for the first time the combined effects of the smoking status and the *ALDH2* genotype on the risk of MI.

2. Methods

A cross-sectional analysis of 396 subjects with CAD was performed, including 221 subjects with MI [144 subjects with acute MI (AMI) and 77 subjects with old MI (OMI)] and 175 subjects with stable angina (SA), as well as 473 age- and sex-matched non-CAD controls. All CAD subjects underwent cardiac catheterization and were found to have ≥75% stenosis in the coronary arteries. Non-CAD controls without a history or electrocardiographic signs of CAD were recruited from a health screening program. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from each participant.

An overweight status was defined as a body mass index (BMI) of ≥25 kg/m^2. Diabetes was defined as a fasting plasma glucose level of ≥7.0 mmol/L or a 2-h level of ≥11.1 mmol/L after a 75 g oral glucose load and a hemoglobin A1c level of ≥6.5% or history of diabetes. Hypertension was defined as a systolic BP of ≥140 mm Hg, diastolic BP of ≥90 mm Hg or history of hypertension. Dyslipidemia was defined as a triglyceride level of ≥1.7 mmol/L, HDL cholesterol level of <1.0 mmol/L or low-density lipoprotein (LDL) cholesterol level of ≥3.6 mmol/L and/or history of dyslipidemia. Smoking was defined as current, past or never based on the patient's smoking status at the time of cardiac catheterization or the health screening.

Genomic DNA was prepared from whole blood using a DNA purification kit (Flexi Gene DNA kit, QIAGEN, Hilden, Germany). The *ALDH2*1/*2* alleles were determined using a real-time TaqMan allelic discrimination assay (Step One Plus Real-Time PCR system version 2.1; Applied Biosystems, Tokyo, Japan) according to the protocols provided by the manufacturer (assay no. C_11703892_10). All reagents were purchased from Applied Biosystems. To ensure the genotyping quality, we included DNA samples as internal controls, hidden samples with known genotypes and negative controls (water).

The data are presented as the mean ± standard deviation or proportion for categorical variables. A Student's *t*-test or one-way analysis of variance and Fisher's exact test were used for comparisons of continuous and categorical variables, respectively. The associations between the *ALDH2* genotype or smoking status and prevalence of MI or SA were calculated as odds ratios (ORs) and 95% confidence intervals (95%CIs) using a logistic regression analysis. The ORs were adjusted for overweight, diabetes, hypertension and dyslipidemia. The interactive and combined effects of smoking status sand the *ALDH2* genotype on the risk of MI or SA were also analyzed using a logistic regression model. The interactive effects of smoking status and the *ALDH2* genotype on the peak creatine kinase (CK) level were also analyzed using a multiple linear regression model in the subjects with AMI. Factors influencing the peak CK level were determined using a multiple linear regression analysis with calculations of the adjusted partial regression coefficient (*B*). In addition, the effects of the interaction between the *ALDH2* genotype and smoking status or alcohol intake on the lipid profiles in the patients with AMI were assessed using multiple linear regression analyses. A p value of <0.05 was considered to be statistically significant. The statistical power at a significance (alpha) level of 0.05 (two-tailed) based on the sample size of this study was calculated using the SamplePower software program (version 2.0). All other statistical analyses were performed using the SPSS software package (version 17.0, IBM Japan Inc. Tokyo, Japan).

3. Results

The clinical characteristics of the subjects are shown in Table 1. The prevalence of cardiovascular risk factors, such as ever-smoking, overweight, diabetes, hypertension and

Table 1
Clinical characteristics of the subjects.

	Control	Myocardial infarction	Stable angina	p
n (male, %)	473 (72.7)	221 (74.7)	175 (71.4)	0.77
Age (years)	68.8 ± 7.6	68.5 ± 10.8	69.5 ± 8.5	0.46[a]
BMI (kg/m^2)	23.1 ± 3.0	24.1 ± 3.3	23.8 ± 3.7	<0.001[a]
Overweight (%)	23.6	38.9	32.0	<0.001
Smokers (past/current, %)	34.2/9.5	44.3/20.8	52.0/13.1	<0.001
Drinkers (%)	57.5	30.3	29.7	<0.001
Systolic BP (mm Hg)	122.9 ± 15.5	127.3 ± 19.5	131.1 ± 17.9	<0.001[a]
Diastolic BP (mm Hg)	72.3 ± 10.1	72.2 ± 13.4	70.1 ± 11.8	0.07[a]
Triglycerides (mmol/L)	1.2 ± 0.6	1.2 ± 0.6	1.3 ± 0.6	0.09[a]
HDL cholesterol (mmol/L)	1.7 ± 0.4	1.2 ± 0.3	1.3 ± 0.4	<0.001[a]
LDL cholesterol (mmol/L)	3.1 ± 0.7	2.2 ± 0.7	2.1 ± 0.6	<0.001[a]
Diabetes (%)	17.3	41.6	53.1	<0.001
Hypertension (%)	46.5	73.3	74.3	<0.001
Dyslipidemia (%)	54.3	79.2	73.7	<0.001
ALDH2 genotype				
**1/*1,*1/*2, *2/*2* (%)	57.5/36.6/5.9	48.9/41.6/9.5	59.4/32.6/8.0	0.10
**2* allele (%)	42.5	51.1	40.6	0.06

The data are the means ± standard deviation or proportions for categorical variables.
[a] Assessed by one-way analysis of variance (otherwise, Fisher's exact test was used).