Original Article

Aldehyde Dehydrogenase 2 Gene Is a Risk Factor for Myocardial Infarction in Japanese Men

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In epidemiological studies, moderate alcohol consumption has been consistently associated with a reduced risk of myocardial infarction (MI). About half of Japanese show an extremely high sensitivity to alcohol (ethanol), which is due to a missense mutation from glutamic acid (Glu) to lysine (Lys) at codon 487 in an isoenzyme of aldehyde dehydrogenase (ALDH2) with a low K_m. We obtained a preliminary result that subjects homozygous for the Lys 487 allele had higher risk for myocardial infarction. The purpose of the present study was to assess this hypothesis by employing a larger cohort of subjects with MI. The experimental group consisted of 342 male subjects with demonstrated MI who were selected randomly from our outpatient clinic. As controls, we employed 1,820 male subjects with no cardiovascular complications who were selected from the Suita Study. All subjects provided their written informed consent to participate in the genetic analyses. Subjects with MI were older and had higher body mass index, higher prevalence of diabetes mellitus, higher prevalence of smoking habit, higher prevalence of the Lys/Lys genotype (homozygous for Lys 487 allele), and lower high density lipoprotein (HDL) cholesterol level (HDL-C). The ALDH2 genotype affected the level of alcohol consumption, and HDL-C. Multiple logistic analyses indicated that the odds ratio of the Lys/Lys genotype to the Lys/Glu+Glu/Glu genotype was 1.56 (p=0.0359). Inclusion of HDL-C as one of the independent variables downplayed the importance of the ALDH2 genotype. This may indicate that the ALDH2 genotype affects MI *via* its effects on HDL-C. In conclusion, the ALDH2 Lys/Lys genotype is a risk factor for myocardial infarction in Japanese men due to its influence on HDL cholesterol level.
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***Key Words*: alcohol, genetics, myocardial infarction, aldehyde dehydrogenase 2, high density lipoprotein cholesterol**

Introduction

In epidemiological studies, moderate consumption of alcohol has been consistently associated with a reduced risk of myocardial infarction (*1*−*4*). The mechanisms underlying this association have not been fully clarified, but may include the effect of alcohol on high density lipoprotein (HDL) cholesterol level (*1*, *2*). About half of Japanese show an extremely high sensitivity to alcohol (ethanol), which is due to a missense mutation from glutamic acid (Glu) to lysine (Lys) at codon 487 in an isoenzyme of aldehyde dehydrogenase

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Table 1. Characteristics of the Study Population

	Control	MI	*p* value
N	1,820	342	
ALDH2	159/786/875	43/139/160	0.0792
Age	60.6 (0.3)	63.7 (0.6)	<0.001
BMI	23.0 (0.06)	23.7 (0.2)	0.0003
Alcohol	0.84 (0.02)	0.78 (0.05)	n.s.
%smoker	40.0	67.4	<0.001
%HT	39.2	55.8	<0.001
%DM	21.2	43.9	<0.001
HbA1c	5.4 (0.03)	5.7 (0.06)	<0.001
FBS	101 (0.6)	117 (1.5)	<0.001
Chol	203 (0.8)	201 (1.8)	n.s.
Trigly	143 (2.5)	142 (5.7)	n.s.
HDL	55 (0.3)	42 (0.8)	<0.001

Values are expressed as the mean±SE. *N*, number of subjects; MI, patients with myocardial infarction; ALDH2, number of subjects according to ALDH2 genotype (Lys/Lys, Lys/Glu, Glu/Glu); Age, years old; BMI, body mass index (kg/m^2); Alcohol, alcohol consumption in cups/day (one cup of Japanese alcohol corresponds to 25.2 ml ethanol); %smoker, percentage of subjects with a smoking habit; %HTN, percentage of subjects with hypertension; %DM, percentage of subjects with diabetes mellitus; HbA1c, shown as %; FBS, fasting blood sugar (mg/dl); Chol, cholesterol (mg/dl); Trigly, triglycerides (mg/dl); HDL, HDL cholesterol (mg/dl).

(ALDH2) with a low K_m. Thus, in Japanese, the level of alcohol consumption, and therefore the level of HDL cholesterol, are greatly influenced by the genotype of ALDH2 (*5–9*). In our previous study, we obtained a preliminary result that the ALDH2 Lys/Lys genotype (homozygous for the Lys 487 allele), which is associated with a lower metabolic rate of acetaldehyde, seems to be a risk factor for myocardial infarction (*9*). The purpose of the present study was to assess this hypothesis by employing a greater number of subjects with myocardial infarction.

Materials and Methods

Subjects

Control subjects were derived from the Suita Study (*9, 10*). The selection criteria and design of the Suita Study have been described previously (*11*). The sample consisted of 14,200 men and women aged 30 to 79 years, stratified by sex and 10-year age groups, who were selected randomly from the municipal population registry. All subjects in the sample were sent a letter inviting them to attend a series of regular follow-up examinations (*i.e.*, one examination every 2 years). The basic sampling of the population started in 1989 with a cohort study base, and 51.7% (n=7,347) of the subjects responded to the invitation letter and had paid their initial visit to the National Cardiovascular Center by February, 1997. The participants visited the National Cardiovascular Center every 2 years for regular health check-ups. For the present study, we selected only male subjects without any cardiovascular complications (control group: n=1,820). The study group consisted of male subjects with documented myocardial infarction who were selected randomly from our outpatient clinic and met the following criteria: 1) chest pain of ≧30 min duration; 2) electrocardiographic ST segment elevation of ≧0.1 mV in two or more leads in the same vascular territory; and 3) subsequent elevation of creatine phosphokinase levels to more than twice the normal range (n=342). All subjects provided their written informed consent to participate in the genetic analyses. The present study was approved by the Committee on Genetic Analysis and Genetic Therapy of the National Cardiovascular Center.

DNA Studies

The ALDH2 genotype was determined by the TaqMan method as previously reported (*9, 10*). Ten nanograms of sample DNA was amplified by PCR according to the manufacturer's recommendations (PE Applied Biosystems, Foster City, USA). The sense and antisense PCR primers and probes for Glu and Lys at codon 487 were as follows:
Sense primer: 5′-gtcaactgctatgatgtgtttgg-3′
Antisense primer: 5′-ccaccagcagaccctcaag-3′
Probe for Glu: Tet-gcaggcatacactgaagtgaaaactgtg
Probe for Lys: Fam-tgcaggcatacactaaagtgaaaactgtg

The genotypes corresponding to homozygosity for the Lys 487 allele, heterozygosity for the Lys 487 allele, and homozygosity for the Glu 487 allele were described as Lys/Lys, Lys/Glu, and Glu/Glu, respectively.

Statistical Analysis

Values are expressed as the mean±SE. All statistical analyses were performed using the JMP and StatView statistical software packages (SAS Inst. Inc., Cary, USA). Multiple linear regression and multiple logistic analyses were performed with other covariates. Differences in numerical data among the groups were analyzed by one-way/two-way analysis of variance (ANOVA) and unpaired Student's *t*-test. Differences in frequencies among the groups were tested by contingency table analysis. In multivariate analyses, genotypes of ALDH2 were categorized into two groups, namely Lys/Lys and Lys/Glu+Glu/Glu. Sample power calculation was performed using SPSS software (SPSS Inc., Chicago, USA).

Results

Subject Characteristics

Table 1 shows the characteristics of the study population.

Table 2. Relationship between Characteristics and ALDH2 Phenotype

	Lys/Lys	Lys/Glu	Glu/Glu	*p* value
N	202	925	1,035	
MI	43	139	160	0.0792
%MI	21.3	15.0	15.5	
Age	61.3 (0.8)	61.5 (0.4)	60.6 (0.4)	n.s.
BMI	23.1 (0.2)	23.0 (0.1)	23.3 (0.1)	n.s.
Alcohol	0.21 (0.06)	0.6 (0.03)	1.16 (0.03)	$<$0.0001
%smoker	48.5	47.9	47.7	n.s.
%HTN	40.6	37.7	46.9	0.0002
%DM	22.8	26.6	23.5	n.s.
HbA1c	5.5 (0.09)	5.6 (0.04)	5.5 (0.04)	n.s.
FBS	103 (2.0)	102 (0.9)	105 (0.9)	0.0397
Chol	203 (2.3)	203 (1.1)	203 (1.0)	n.s.
Trigly	134 (7.4)	137 (3.5)	150 (3.3)	0.0121
HDL	48 (1.0)	52 (0.5)	54 (0.5)	$<$0.0001

Values are expressed as the mean$\pm$SE. *N*, number of subjects according to ALDH2 genotype (Lys/Lys, Lys/Glu, Glu/Glu); MI, patients with myocardial infarction; Age, years old; BMI, body mass index (kg/m^2); Alcohol, alcohol consumption in cups/day (one cup of Japanese alcohol corresponds to 25.2 ml ethanol); %smoker, percentage of subjects with a smoking habit; %HTN, percentage of subjects with hypertension; %DM, percentage of subjects with diabetes mellitus; HbA1c, shown as %; FBS, fasting blood sugar (mg/dl); Chol, cholesterol (mg/dl); Trigly, triglycerides (mg/dl); HDL, HDL cholesterol (mg/dl).

Table 3. Multiple Logistic Analyses

	Odds ratio	*p*
A. Multiple logistic analyses		
ALDH2	1.56 (1.022−2.35)	0.0359
Smoker	16.51 (11.51−24.36)	$<$0.0001
DM	2.28 (1.735−2.998)	$<$0.0001
B. Multiple logistic analyses including HDL cholesterol		
ALDH2	1.13 (0.707−1.795)	0.5926
Smoker	11.9 (8.083−18.048)	$<$0.0001
DM	2.13 (1.563−2.890)	$<$0.0001

ALDH2: in multivariate analyses genotypes of ALDH2 were categorized into two groups, namely Lys/Lys and Lys/Glu+Glu/Glu. Smoker, subjects with smoking habit; DM, presence of diabetes mellitus.

Table 4. Multiple Logistic Analyses in Younger Subjects

	Odds ratio	*p*
A. Multiple logistic analyses in younger subjects ($<$60 years old)		
ALDH2	2.01 (1.154−3.443)	0.012
Smoker	18.64 (10.89−34.53)	$<$0.0001
DM	2.70 (1.862−3.909)	$<$0.0001
B. Multiple logistic analyses in younger subjects ($<$60 years old) including HDL cholesterol		
ALDH2	1.36 (0.720−2.497)	0.3364
Smoker	12.7 (7.139−24.340)	$<$0.0001
DM	2.17 (1.414−3.312)	0.0004

ALDH2: in multivariate analyses genotypes of ALDH2 were categorized into two groups, namely Lys/Lys and Lys/Glu+Glu/Glu. Smoker, subjects with smoking habit; DM, presence of diabetes mellitus.

Subjects with myocardial infarction were older, had higher blood sugar and HbA1c levels, lower HDL cholesterol level, higher body mass index (BMI), higher prevalence of diabetes mellitus (DM), higher prevalence of smoking habit, higher prevalence of hypertension and a higher prevalence of the ALDH2 Lys/Lys genotype.

Significance of the ALDH2 Genotype in Myocardial Infarction

Table 2 shows characteristics of subjects according to the ALDH2 genotype. The ALDH2 genotype affected the level of alcohol consumption, triglyceride level, HDL cholesterol level, blood sugar level and prevalence of hypertension.

The prevalence of subjects with myocardial infarction tended to be higher in subjects with the ALDH2 Lys/Lys genotype (p=0.0792, chi-squared test). Multiple logistic analyses indicated that the odds ratio of the risk of myocardial infarction for the Lys/Lys genotype compared with the Lys/Glu+Glu/Glu genotypes was 1.56 (p=0.0359; 95% confidence interval (CI): 1.02−2.35; Table 3A). Other variables were age ($p<0.0001$), BMI ($p<0.0001$), smoking habit ($p<0.0001$) and DM ($p<0.0001$) (Table 3A). Inclusion of HDL cholesterol as one of the independent variables downplayed the importance of the ALDH2 genotype (p=0.5926; 95% CI: 0.71−1.80; Table 3B). *P*-values for age, HDL cholesterol and BMI were <0.0001, <0.0001 and 0.1399, respectively. The association of the ALDH2 Lys/Lys genotype with myocardial infarction was more evi-

Table 5. Determinants of HDL Cholesterol

	p
A. Determinants of HDL cholesterol	
ALDH2	<0.0001
Smoker	<0.0001
DM	0.0022
B. Determinants of HDL cholesterol including alcohol consumption	
ALDH2	0.0014
Smoker	<0.0001
DM	0.0070
Alcohol	<0.0001

ALDH2: in multivariate analyses genotypes of ALDH2 were categorized into two groups, namely Lys/Lys and Lys/Glu+Glu/Glu. Smoker, subjects with smoking habit; DM, presence of diabetes mellitus; Alcohol, alcohol consumption.

dent in younger subjects (<60 years old). Multiple logistic analysis indicated that the odds ratio of the Lys/Lys genotype to the Lys/Glu+Glu/Glu genotypes was 2.01 (p=0.0120; 95% CI: 1.15–3.44; Table 4A). Other variables were age (p<0.0001), BMI (p=0.0006), smoking habit (p<0.0001) and DM (p<0.0001) (Table 4A). Again, inclusion of HDL cholesterol as one of the independent variables downplayed the importance of the ALDH2 genotype (p=0.3364; 95% CI: 0.72–2.50; Table 4B). *P*-values of age, HDL cholesterol and BMI were <0.0001, <0.0001 and 0.7142, respectively. These results suggest that the ALDH2 genotype may affect myocardial infarction by affecting HDL cholesterol.

Determinants of HDL Cholesterol

Multiple regression analysis indicated that the level of HDL cholesterol was determined by the ALDH2 genotype (Lys/Lys=1, Lys/Glu+Glu/Glu=2), presence of DM, and presence of smoking habit (Table 5A). Inclusion of alcohol consumption as one of the independent variables downplayed the importance of the ALDH2 genotype.

Sample Power Calculation

The sample power calculation in the present study indicated that sample power was 0.59 for the distribution, sample size and α value (0.05, two-tailed).

Discussion

Light-to-moderate consumption of alcohol has been consistently associated with a reduced risk of myocardial infarction (*1–4*). Heavier alcohol consumption, in contrast, is associated with no change or even an increase in this risk (*2*). The mechanisms underlying the former, beneficial association have not been fully clarified, but may include the effect of alcohol on HDL cholesterol level (*1*, *2*, *12–14*). Recently, Hines *et al*. reported that moderate drinkers who are homozygous for the slow-oxidizing alcohol dehydrogenase 3 (ADH3) allele ($\gamma 2 \gamma 2$) have higher HDL cholesterol levels and a substantially decreased risk of myocardial infarction (*1*). Since the predominant function of ADH3 is to metabolize ethanol to acetaldehyde, they suggested that a slower rate of clearance of ethanol enhances the beneficial effect of moderate alcohol consumption on the risk of cardiovascular disease. In the present study, we showed that subjects who were homozygous for the Lys allele of ALDH2 had lower HDL cholesterol levels and a substantially increased risk of myocardial infarction. Since the ALDH2 enzyme metabolizes acetaldehyde to acetic acid, individuals with the Lys allele of ALDH2, which is associated with a lower metabolic rate of acetaldehyde, can tolerate only very small amounts of alcohol. The determinants of HDL cholesterol were the ALDH2 genotype (Lys/Lys=1, Lys/Glu+Glu/Glu=2), presence of DM, and presence of smoking habit. However, inclusion of alcohol consumption as one of the independent variables downplayed the importance of the ALDH2 genotype. Thus, since alcohol consumption determined by the ALDH2 genotype affects HDL cholesterol level, we emphasized the importance of the ALDH2 genotype for risk of myocardial infarction (*8*, *9*).

De Oliveira e Silva *et al*. reported the mechanism of the effect of alcohol on HDL cholesterol level. They demonstrated that ethanol intake results in dose-dependent increases in plasma concentrations of the major HDL components (HDL cholesterol, apolipoproteins A-I and -II (apoA-I and -II) through an increase in the HDL apolipoprotein transport rate without a change in fractional catabolic rate or HDL particle size (*12*). However, the molecular mechanism of the increased apolipoprotein synthesis is unclear in humans *in vivo*. In hepatocyte culture, the effect of ethanol on apolipoprotein synthesis appears to involve the microsomal ethanol-oxidizing system and is speculated to be due to intracellular increases in phospholipid and cholesterol (*15*, *16*).

We have previously assessed the significance of genetic polymorphisms of angiotensin converting enzyme (I/D), endothelial nitric oxide synthase (T(−788)C) and ABCA1 genes in ischemic heart disease using the same study population (*10*, *17*). Our results indicated that these polymorphisms were not associated with ischemic heart disease in our study population, a finding that was discrepant with those of other previous studies (*18–20*). We have frequently encountered such discrepancy among association studies (*21–28*). The reason for the discrepancy is unclear, but it may be related to low statistical power or to differences among the ethnic groups studied.

The sample power was 0.59 in the present study, meaning that 59% of studies would be expected to yield a significant effect, rejecting the null hypothesis that the odds ratio is 1.0. To increase the sample power, additional studies employing larger cohorts will be needed.

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