

Original Article

Genetic Association between Aldehyde Dehydrogenase 2 (ALDH2) Variation and High-Density Lipoprotein Cholesterol (HDL-C) Among Non-Drinkers in Two Large Population Samples in Japan

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Aim: Moderate alcohol consumption appears to confer some protection against coronary heart disease, which is related to an increase in high-density lipoprotein cholesterol (HDL-C). The genotype of aldehyde dehydrogenase 2 (ALDH2) is closely related to alcohol metabolism but a relationship between ALDH2 genotypes and HDL-C levels has not been proven. We undertook a large-scale correlation study between HDL-C levels and ALDH2 genotype among Japanese non-drinkers to investigate the possibility that HDL-C levels could be associated with ALDH2 genotype.

Methods: We examined a population-based sample of Japanese subjects who do not consume alcohol ($n=1,736$) to investigate the relationship between ALDH2 genotypes and lipid or lipoprotein concentrations in serum. We also investigated whether an association between ALDH2 genotype and HDL-C levels might be found in another Japanese sample.

Results: In an independent population of non-drinkers from a different geographical region of Japan, HDL-C levels were associated with the same ALDH2 genotypes.

Conclusions: The results of the present study suggested that genetic variation in the ALDH2 gene can influence HDL-C levels, independent of alcohol consumption.

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Key words; Single nucleotide polymorphism, Aldehyde dehydrogenase 2, High-density lipoprotein, Association study

Introduction

Hyperlipidemia is a common multifactorial disease, with onset and progression determined by genetic and environmental factors¹⁻³. Over the past two

decades or more, abundant evidence has accumulated from clinical, epidemiological, and experimental studies to suggest that lipid and lipoprotein concentrations in plasma are closely associated with genetic factors^{4,5}. Clarification of genetic risk is essential for the prevention and early treatment of hyperlipidemia.

Much attention has been focused lately on the high-density lipoprotein (HDL) system because of its close, inverse relationship to the incidence of cardiovascular disease⁶⁻¹³. In addition, many epidemiological studies have shown that low HDL-C as a risk factor for atherosclerosis¹⁴. An association of dietary

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habits with HDL-C levels has also been indicated; on observational grounds, for example, moderate alcohol consumption has been related to protection against ischemic heart disease. This effect is usually attributed to alcohol-induced increases in HDL-C concentrations^{15, 16}. Despite the importance of a likely relationship between alcohol consumption and increased HDL-C levels, the mechanisms involved have not been clarified.

ALDH2 converts acetaldehyde into acetate, playing a crucial role in the oxidation of acetaldehyde¹⁷. A point mutation (G to A transition in exon¹²) of ALDH2 at position 1510 (Glu487Lys amino-acid variation of the protein) results in an inactive enzyme¹⁸. Approximately four in ten East-Asians show low or absent ALDH2 enzymatic function; this situation tends to bring about accumulation of acetaldehyde, especially after alcohol intake¹⁹. Although potential relationships between the ALDH2 genotype and response to alcohol, and between alcohol intake and HDL-C levels, have drawn much attention, ALDH2 may in fact play fundamental physiological roles other than just participation in alcohol metabolism. Indeed, various aldehydes are widespread in the human body, being produced during bio-transformation of various endogenous metabolites such as lipids, amino acids, and their derivatives. Since acetaldehyde is cytotoxic, a disturbed acetaldehyde metabolism might be a factor in some types of human pathophysiology.

To investigate the possibility that HDL-C levels could be associated with the ALDH2 genotype regardless of alcohol intake, we undertook a large-scale correlation study between HDL-C levels and the ALDH2 genotype in two independent Japanese populations consisting exclusively of non-drinkers. Our results in the first cohort and their validation in the second demonstrated that the ALDH2 genotype indeed can influence HDL-C levels in the Japanese population, even in the absence of alcohol intake.

Materials and Methods

Study Populations

Takahata (T) population: The present study is part of an ongoing Molecular Epidemiological Study that utilizes the Regional Characteristics of a 21st Century Center of Excellence (COE) Program in Japan. Details of this population cohort have been reported previously²⁰. In brief, this community-based group represented the general population from 40 to 87 years of age, living in Takahata, Yamagata Prefecture, Japan. Of the 1,736 non-drinking subjects enrolled in the present study, 359 were men (mean age \pm SD, 66.7 \pm

10.5) and 1,377 were women (mean age \pm SD, 63.8 \pm 9.8).

Funagata (F) population: The Funagata population sample was described previously²¹. All individuals older than 35 years residing in the town of Funagata were registered. Of the 774 non-drinking subjects enrolled in the present genetic investigation, 119 were men (mean age 65.5 \pm 11.4), and 655 were women (63.1 \pm 11.9).

Utah (U) population: 1,029 subjects (488 men and 541 women) from the state of Utah, USA were randomly sampled to represent a general Caucasian population.

Informed consent was obtained from each subject after a full explanation of the study had been given. The physical and clinical profiles of these subjects (age, gender, and plasma lipoprotein levels) were recorded. Absence of alcohol intake was confirmed by a questionnaire.

DNA Extraction and SNP Genotyping

Genomic DNA was extracted from white blood cells using a Genomic DNA purification kit (Talent, Trieste, Italy). Extracted DNA was dissolved in a TE buffer (10 mmol/L Tris-HCl, pH 8.0, 1 mmol/L EDTA) and stored at -20°C until use. Nine single-nucleotide polymorphisms (SNPs) within the ALDH2 gene (rs4767944, rs11609628, rs4648328, rs10849970, rs671, rs2158029, rs11066028, rs11066029, and rs10849971) derived from the dbSNP database of the NCBI (<http://www.ncbi.nlm.nih.gov/SNP/>) were genotyped on the basis of minor allele frequencies (MAF > 0.1) in our Japanese study populations. Genotyping was performed by Invader assay (Third Wave Technologies, Madison, WI)^{22, 23} and the TaqMan allelic discrimination assay²⁴.

Statistical Analyses

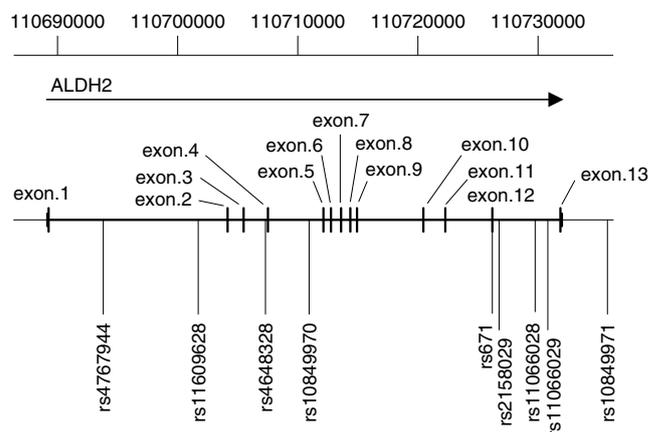
Values are expressed as the means \pm SE. The Statistical Package for the Social Sciences (SPSS Inc., ver. 15.0) was used for all analyses. Quantitative associations between genotypes and plasma lipid levels (mg/dL) were examined by analysis of variance (ANOVA), with regression analysis as a post-hoc test. The Hardy-Weinberg equilibrium of alleles at each locus was evaluated using a chi-squared test as implemented in the R 2.5.0 package "genetics" (<http://www.r-project.org/>). Significant levels of association were defined when the given p value of the ANOVA F -test was < 5% ($p < 0.05$).

Table 1. Clinical characteristics of Takahata and Funagata study groups (non-drinkers)

Measures	Takahata (<i>n</i> = 1,736)	Funagata (<i>n</i> = 774)
Age (y)	64.4 (0.2)	63.4 (0.4)
Male/Female	359/1,377	119/655
Total cholesterol (mg/dL)	204.7 (0.7)	205.7 (1.2)
Triglycerides (mg/dL)	100.5 (1.2)	106.3 (2.5)
HDL cholesterol (mg/dL)	59.0 (0.3)	59.2 (0.5)
LDL cholesterol (mg/dL)	128.5 (0.7)	NE

Values are percentage, unadjusted means (SE).

Abbreviations: y, years; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NE, not examined.

**Fig. 1.** Exon-intron structure of ALDH2.

The locations of nine tested variations are indicated.

Table 2. Summary of genotyping data for polymorphisms in the ALDH2 gene of non-drinking Takahata subjects

NCBI ref.ID ^a	Chromosome ^a	Chromosome ^a Position	Major ^a allele	Minor ^a allele	Allele frequency ^b
rs4767944	12	110693724	T	C	0.61/0.39
rs11609628	12	110701591	G	A	0.79/0.21
rs4648328	12	110707171	C	T	0.79/0.21
rs10849970	12	110710816	A	G	0.61/0.39
rs671	12	110726149	G	A	0.67/0.33
rs2158029	12	110726588	A	G	0.60/0.40
rs11066028	12	110729553	C	A	0.82/0.18
rs11066029	12	110730584	G	A	0.60/0.40
rs10849971	12	110735580	G	A	0.60/0.40

^aDatabase ID for SNP of the National Center for Biotechnology Information (homepage: <http://www.ncbi.nlm.nih.gov/>) (Build = 36.2).

^bNon-drinking subjects of the Takahata study were genotyped (*n* = 1,736).

Results

Clinical Characteristics of Non-Drinkers in the Takahata (T) and Funagata (F) Study Groups

Relevant baseline characteristics of two non-drinking populations, Takahata (T) (*n* = 1,736) and Funagata (F) (*n* = 774), are shown in **Table 1**.

Association of a Polymorphism in ALDH2 with Plasma HDL-C Levels

To carry out correlation analyses we first estimated allelic frequencies and heterozygosities for all nine SNPs in ALDH2 (**Fig. 1**) by genotyping the 1,736 individuals of the T cohort (**Table 2**). When these subjects were genotypically categorized into three groups for each of the nine SNPs, we detected no deviation of genotype frequencies from Hardy-Weinberg equilibrium.

Of the four lipoprotein parameters analyzed (TC,

TG, LDL-C, and HDL-C), we detected a significant correlation only between genotypes of the Glu487Lys variation (rs671 G/A) and plasma concentrations of HDL-C ($p = 0.0014$; **Table 3**). HDL-C levels among the three genotypic categories, i.e., 943 homozygous Glu allele carriers (mean HDL-C, 60.2 ± 0.5 mg/dL), 702 heterozygous carriers (57.7 ± 0.5 mg/dL), and 91 homozygous Lys allele carriers (57.9 ± 1.4 mg/dL), indicated a dominant effect of the Lys allele in lowering HDL-C (**Table 4**). No association was detected for plasma levels of TC, TG, or LDL-C, or for the other eight SNPs.

When the Takahata subjects were separated into those who carried at least one Lys allele (Glu/Lys and Lys/Lys) and those with none (Glu/Glu), the former group showed significantly lower plasma HDL-C levels (57.7 ± 1.5 mg/dL versus 60.2 ± 0.5 mg/dL) ($p = 0.0029$).

Table 3. Correlation between variants of rs671 and neighboring SNPs with lipid phenotypes in non-drinking Takahata subjects

	NCBI reference ID	TC	TG	LDL-C	HDL-C
1	rs4767944	NS	NS	NS	NS
2	rs11609628	NS	NS	NS	NS
3	rs4648328	NS	NS	NS	NS
4	rs10849970	NS	NS	NS	NS
5	rs671	NS	NS	NS	0.0014
6	rs2158029	NS	NS	NS	NS
7	rs11066028	NS	NS	NS	NS
8	rs11066029	NS	NS	NS	NS
9	rs10849971	NS	NS	NS	NS

P-value was calculated for regression analysis with ANOVA *F*-test. Abbreviations: TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NS, not significant

Table 4. Association between HDL-C and ALDH2 polymorphisms analyzed at the rs671 and neighboring SNPs in non-drinkers

SNP No.	NCBI ref.ID	HDL-C levels (mean \pm SE)			<i>p</i> -value
1	rs4767944	CC	TC	TT	NS
		60.9 \pm 1.0	59.2 \pm 0.5	58.3 \pm 0.5	
2	rs11609628	AA	AG	GG	NS
		58.5 \pm 2.8	59.9 \pm 0.7	58.8 \pm 0.4	
3	rs4648328	CC	TC	TT	NS
		58.8 \pm 0.4	59.9 \pm 0.7	58.2 \pm 2.6	
4	rs10849970	AA	GA	GG	NS
		58.3 \pm 0.5	59.3 \pm 0.5	60.9 \pm 1.1	
5	rs671	GG	AG	AA	0.0014
		60.2 \pm 0.5	57.7 \pm 0.5	57.9 \pm 1.4	
6	rs2158029	AA	GA	GG	NS
		58.3 \pm 0.5	59.2 \pm 0.5	60.9 \pm 1.0	
7	rs11066028	AA	CA	CC	NS
		61.4 \pm 4.1	59.6 \pm 0.7	58.9 \pm 0.4	
8	rs11066029	AA	GA	GG	NS
		60.7 \pm 1.0	59.2 \pm 0.5	58.4 \pm 0.5	
9	rs10849971	AA	GA	GG	NS
		60.9 \pm 1.0	59.3 \pm 0.5	58.3 \pm 0.5	

P-value was calculated for regression analysis with ANOVA *F*-test. Abbreviations: SNP, single nucleotide polymorphism; No., number; ref., reference; HDL-C, high-density lipoprotein cholesterol; NS, not significant

Association of the 487Lys Allele with HDL-C in the Funagata (F) Population

To examine the reproducibility of our findings, we examined a second cohort (F) of the Japanese non-drinking population that had been independently en-

rolled elsewhere in Japan. Analysis of variance in these subjects confirmed a significant correlation between the ALDH2 Lys allele and HDL-C levels; i.e., it was lower among 487Lys homozygotes (mean \pm SE, 56.9 \pm 2.6 mg/dL) and heterozygotes (57.5 \pm 0.9 mg/dL) than among 487Glu homozygotes (60.5 \pm 0.7 mg/dL) (p = 0.014).

Since the rs671 site of ALDH2 was monomorphic in the Utah Caucasians (all 1,029 individuals in that cohort were of the G/G genotype), it was impossible to correlate the gene with lipoprotein profiles in that ethnic group.

Discussion

This study was designed to evaluate the possible association of polymorphisms within the ALDH2 gene with HDL-C concentrations in the plasma of a non-drinking population. In recent epidemiological studies, much attention has been given to a relationship between HDL-C and alcohol intake; however, a potential connection between ALDH2 genotype and HDL-C concentration has never been thoroughly clarified as to whether any ALDH2 variation is directly associated with HDL-C levels in the absence of alcohol intake.

We have identified a significant correlation between plasma HDL-C and a variation in exon 12 of the ALDH2 gene (SNP rs671, G/A; Glu487Lys) in two independent Japanese populations. No other SNPs in this gene that were examined in the present study showed any correlation with HDL-C levels. The 487Lys amino-acid variant was associated with reduced activity of the enzyme, suggesting that this functional site could be responsible for the variations in plasma HDL-C levels seen among non-drinkers. The positive association observed in the initial population (T), consisting of 1,736 non-drinkers, was confirmed in another Japanese population (F), consisting of 774 non-drinkers.

Over the past two decades or more, epidemiological investigations have shown consistently that moderate alcohol consumption is associated with a reduced risk of myocardial infarction^{25, 26}. The apparent benefit of alcohol consumption in reducing the risk of myocardial infarction has been attributed primarily to an increase in HDL-C in plasma²⁶⁻²⁸. We have demonstrated here that ALDH2 487Lys is associated with low HDL-C levels in the absence of alcohol intake in the Japanese population.

The molecular mechanisms whereby the Glu487Lys variation in ALDH2 might affect plasma HDL-C levels require clarification; for example, ALDH2 appears to protect against oxidative stress²⁹.

In one recent study, a deficiency of mitochondrial ALDH2 resulted in elevation of serum lipid peroxides in a community-dwelling female population, an indication that ALDH2 deficiency can enhance oxidative stress *in vivo*³⁰. Furthermore, several other investigators have shown that consumption of polyphenols, known for their antioxidant properties, increases the antioxidative capacity of both plasma and serum toward HDL^{31, 32}. Homozygosity for ALDH2 487Glu, a feature of the Caucasian population examined here, might decrease oxidative stress and thereby improve HDL-C levels *in vivo*.

The present study confirmed that no variants of ALDH2 were detectable in the Caucasian population described in a previous study³³. It is noteworthy that the Glu487Lys variant is almost exclusively present in East Asians, but its selective presence is puzzling. A higher level of acetaldehyde does appear to protect against some diseases that are endemic in East Asia, such as infection by *Entamoeba histolytica*³⁴. Indeed, a specific ALDH inhibitor, nitroimidazole, effectively protects against infection by anaerobic and microaerophilic parasites³⁴.

Since this study was a cross-sectional design, we cannot infer a causal relationship between the ALDH2 genotype and the risk of subsequent ischemic heart disease or stroke, and we have not provided clear evidence that homozygosity for ALDH2 487Glu confers protection against these conditions. Extensive prospective studies will be necessary to establish a link between the ALDH2 genotype and ischemic heart disease in non-drinking populations. Objective data concerning alcohol intake would have improved the reliability of the results reported here; however, we relied on information obtained through questionnaires since that is an accepted method in human epidemiology.

Other enzymes associated with aldehyde metabolism also need to be studied; alcohol dehydrogenase 2 (ADH2), for example. ADH2 polymorphisms are also present among Japanese; in one study, 85% of Japanese subjects possessed the ADH2*2 allele³⁵. Since many of the subjects in our present study possessed an ADH isozyme encoded by the ADH2*2 allele, any effect of ADH2 genotype on alcohol metabolism may have been small in our sample populations; however, extensive investigation of other related genes, ideally in a prospective design including a large population, will be required for comprehensive understanding of human genetic variations as they may relate to HDL-C metabolism.

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