ASSOCIATION OF AN ALDEHYDE DEHYDROGENASE 2 (ALDH2) GENE POLYMORPHISM WITH HYPER-LOW-DENSITY LIPOPROTEIN CHOLESTEROLEMIA IN A JAPANESE POPULATION

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Objective: The relationship among alcohol metabolism, lipid profile and cardiovascular disease has been a matter of concern, and aldehyde dehydrogenase-2 (ALDH2) is one of the key enzymes involved in alcohol metabolism. The frequency of ALDH2 gene G/A polymorphism (with the substitution of glutamic acid to lysine) varies widely among ethnic groups; the polymorphism is prevalent among Asian people but rare in other ethnic groups. The objective of our study was to investigate the association between the ALDH2 gene G/A polymorphism and lipid profile, including the low-density lipoprotein cholesterol (LDL-C) status, in a general Japanese population with no or light-to-moderate alcohol drinking habits.

Methods: Anthropometric and biochemical variables including lipid- and glucose-related factors were measured in a total of 383 Japanese participants (170 males and 213 females; mean age, 45 ± 8.6 years), free of cardiovascular disease. All participants were genotyped by an allele-specific DNA assay.

Results: The numbers of participants with the G/G, G/A and A/A genotypes were 213, 139 and 31, respectively. The percentages of hyper-LDL-cholesterolemia (identified by LDL-C ≥ 3.63 mmol/L) were 31.9%, 45.3% and 29.0% in participants with the G/G, G/A and A/A genotypes, respectively. Carrying the G/A + A/A genotype was a significant and positive factor related to hyper-LDL-cholesterolemia with an odds ratio of 1.62 (95% CI: 1.04–2.52) after adjusting for the other variables including drinking status.

Conclusion: Our findings suggest that the ALDH2 gene G/A polymorphism can affect the lipid profile such as LDL-C status in this population. The association between the polymorphism and LDL-C status warrants further investigation. (*Ethn Dis.* 2012;22[3]:324–328)

Key Words: High-density Lipoprotein, Low-density Lipoprotein, Oxidation, Detoxification, Alcohol

INTRODUCTION

Cardiovascular disease and its risk factors (ie, lipid/lipoprotein disorder) are caused by multiple environmental and genetic components.[1] Since light-to-moderate alcohol consumption reportedly has beneficial effects on the development of coronary artery disease, and this association can be mediated through a favorable lipid/lipoprotein profile, such as an increase in the high-density lipoprotein cholesterol (HDL-C) concentration,[2] special attention has been given to the relationship between cardiovascular disease and genetic polymorphisms affecting alcohol metabolism.[2] Mitochondrial aldehyde dehydrogenase-2 (ALDH2) is one of the key enzymes involved in alcohol metabolism, and is largely responsible for the detoxification of aldehydes generated by alcohol consumption, converting them to acetic acid.[3] A mutation leading to a G to A transition in exon 12 within the ALDH2 gene (resulting in an amino-acid change of glutamic acid to lysine) leads to a slower metabolic function of this enzyme, so Asian people with this mutated polymorphism (present in ~50% of the population) show flushing symptoms (so-called 'Asian flush' or 'Asian glow') due to a high acetaldehyde concentration after drinking.[3]

Limited studies have reported a significant and positive association of the ALDH2 gene A allele with myocardial infarction.[4,5] This association was reportedly independent of alcohol consumption.[5] In addition, the association was dependent on the HDL-C concentration,[4] but not consistently observed.[5] Although the involvement of the G/A polymorphism in the lipid/lipoprotein profile is of interest, only a few studies have investigated this association in the general population.[6–9] On the whole, participants with the A allele exhibited a lower HDL-C concentrations than those without the A allele in non- or

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Table 1. Clinical characteristics according to ALDH2 genotype, *n* (%), mean ± SD or median (interquartile range)

Genotype	G/G	G/A	A/A	*P*
All participants (male, *n*)	213 (100%)	139 (57%)	31 (13%)	-
Age, years	45 ± 8	44 ± 9	47 ± 10	.239
Body mass index, kg/m^2	22.5 ± 3.2	22.7 ± 2.9	23.1 ± 2.6	.508
Drinkers[a]	56 (26.3%)	33 (23.7%)	6 (19.3%)	.830
Current smokers	49 (23.0%)	28 (20.1%)	4 (12.9%)	.400
LDL-cholesterol, mmol/L	2.56 ± .88	2.61 ± .85	2.55 ± .80	.831
Hyper-LDL-cholesterolemia	68 (31.9%)	63 (45.3%)[c]	9 (29.0%)	.026[b]
HDL-cholesterol, mmol/L	1.78 ± .54	1.63 ± .44[d]	1.73 ± .38	.020[b]
Hypo-HDL-cholesterolemia	20 (9.4%)	19 (13.7%)	0 (0.0%)	.064
Triglyceride, mmol/L	.98 (.62–1.56)	.98 (.69–1.51)	.96 (.67–1.29)	.667
Hypertriglyceridemia	48 (22.5%)	27 (19.4%)	5 (16.1%)	.620
Hemoglobin A1c, %	5.1 ± .2	5.1 ± .3	5.1 ± .4	.099

The *P* was calculated in trend by one-way ANOVA for continuous variables, and the between-group difference was examined by the post-hoc multi-comparison test. Because of the skewed distribution, the triglyceride was log-transformed in the analysis. For categorical variables, the trend was calculated by the χ^2-test.

ALDH: aldehyde dehydrogenase, LDL: low-density lipoprotein, HDL: high-density lipoprotein.

[a] Drinkers were defined as individuals who consumed 1 to 3 go/day on average.

[b] $P \leq .05$.

[c] $P = .011$: G/A vs G/G by the residual analysis.

[d] $P = .015$. G/A vs G/G by the post-hoc multi-comparison test.

light-to-moderate drinkers,[6,9] and this association became unclear in those who were more than moderate drinkers.[6,7] We have also noted that individuals with the A allele had a higher concentration of total cholesterol despite the lower concentration of HDL-C in non- or light-to-moderate drinkers in the general Japanese population.[6] The phenomenon may reflect the polymorphic effects on low-density lipoprotein cholesterol (LDL-C) levels, although this point has not yet been discussed. Therefore, the aim of our study was to examine the association between the ALDH2 gene G/A polymorphism and the lipid/lipoprotein profile, including LDL-C status in particular, in a general population with non- or light-to-moderate alcohol drinking habits.

PARTICIPANTS AND METHODS

Study Population

Our study enrolled 383 asymptomatic community-dwelling Japanese participants (170 males and 213 females; mean age, 45 ± 8.6 years), who had no known history of cardiovascular or cerebrovascular disease and who were not taking any medication. None were alcohol-abusers or heavy drinkers. The study was approved by the ethics committee of Kyoto Medical Center and each participant gave informed consent.

Measurements

The smoking and alcohol drinking habits were determined based on self-reported questionnaires and medical interviews. Smokers were defined as current smokers. The consumption of alcohol is often expressed using the go (unit) in Japan. One go contains 23 g of ethanol; drinkers were defined as individuals who consumed 1 to 3 go/day on average; and heavy drinkers (>3 go/day) were excluded from the study. Participant's body mass index (BMI) was calculated (in light clothing) as the weight (kg) divided by the square of the height (m). The fasting serum triglyceride (TG) level was enzymatically measured, and LDL-C and HDL-C levels were measured using homogeneous enzymatic methods (Kyowa Medex Co. Ltd., Tokyo, Japan). Hemoglobin A1c (HbA1c) was measured by a high performance liquid chromatography method (Tosoh Co. Ltd., Tokyo, Japan). Hypertriglyceridemia was identified by TG≥1.69 mmol/L, hyper-LDL-cholesterolemia by LDL-C≥3.63 mmol/L and hypo-HDL-cholesterolemia by HDL-C<1.04 mmol/L in males and <1.29 mmol/L in females, based on the clinical evaluation.[10] DNA was extracted from peripheral blood leukocytes, and genotypes were determined by an intercalater-mediated fluorescent allele-specific PCR method (Toyobo Gene Analysis Co. Ltd., Tsuruga, Japan) using the primers described previously.[6]

Statistical Analyses

Values are given as the means ± standard deviation or medians plus interquartile range. The genotype and allele frequencies for Hardy-Weinberg equilibrium were examined using the χ^2-test. Continuous variables or categorical variables between the groups were compared using one-way ANOVA with the post-hoc multi-comparison test or the χ^2-test with a residual analysis. Because of the skewed distribution, the TG was log-transformed in the analysis. The explanatory variables, such as age, sex, BMI, current smoking and drinking status, lipid profile, HbA1c and genotype, were entered into a logistic regression model to estimate an odds ratio (OR) and 95% confidence interval (95% CI) of hyper-LDL-cholesterolemia or hypo-HDL-cholesterolemia as a criterion variable. The multiple-adjusted logistic regression model analysis was performed after combining the G/A genotype with the A/A genotype to form a single group expressing the A allele, due to a small *n* of A/A genotype participants. In all analyses, $P \leq .05$ was considered to be significant.

RESULTS

The studied participants' characteristics are shown in Table 1. The number of participants with the G/G,

G/A and A/A genotypes was 213, 139 and 31, respectively; these distribution percentages were similar to those described in the previous reports.[4,6,8,9] The frequency of the A-allele was 26.2%. These frequencies were in Hardy-Weinberg equilibrium.

Significant differences were observed in the prevalence of hyper-LDL-cholesterolemia and the levels of HDL-C according to genotypes (Table 1). Participants with the G/A genotype had significantly higher percentages of hyper-LDL-cholesterolemia than those with the G/G genotype. Participants with the A allele had lower HDL-C levels than those without the A allele, and in particular, those with the G/A genotype had significantly lower HDL-C levels than those with the G/G genotype. Differences between sexes and smoking habits were: current male smokers - 46 (46.0%), 26 (45.6%) and 4 (30.8%) with the G/G, G/A and A/A genotypes, respectively, compared to current female smokers - 3 (2.7%), 2 (2.4%) and 0 (0.0%) with the G/G, G/A and A/A genotypes, respectively (data not shown) Likewise the differences between the sexes and alcohol drinking were: current male drinkers - 46 (46.0%), 25 (43.9%) and 6 (46.2%) with the G/G, G/A and A/A genotypes, respectively, compared to female drinkers - 10 (8.8%), 8 (9.8%) and 0 (0.0%) with the G/G, G/A and A/A genotypes (data not shown). However, there was no significant difference in the prevalence of current smokers and drinkers by genotype in males and females.

The results of the multiple-adjusted logistic regression model analysis of hyper-LDL-cholesterolemia are shown in Table 2. Age, male sex, BMI and carrying the G/A + A/A genotype were independently, significantly and positively associated with hyper-LDL-cholesterolemia, while drinking habits were an independently significant variable inversely associated with hyper-LDL-cholesterolemia. The same multiple-adjusted logistic regression model analysis of hyper-LDL-cholesterolemia was performed in the subgroups of non-drinkers or drinkers. As shown in Table 3, age, male sex and carrying the G/A + A/A genotype were independently, significantly and positively associated with hyper-LDL-cholesterolemia in non-drinkers. In the subgroup of drinkers, only non-significant variables, including the G/A+ A/A genotype, were found to be associated with hyper-LDL-cholesterolemia.

In addition, the multiple-adjusted logistic regression model analysis of hypo-HDL-cholesterolemia was performed based on the genotypes (data not shown). The variables that were independently and significantly associated with hypo-HDL-cholesterolemia were as follows: BMI (OR 1.11 [95%CI 1.00–1.24], $P=.05$), hypertriglyceridemia (7.80 [3.47–17.57], $P<.0001$). The genotypes

Table 2. Multivariate-adjusted logistic regression analysis for hyper-LDL-cholesterolemia by genotype

	Odds ratio (95% CI)	*P*
Age, years	1.06 (1.03–1.09)	<.001[b]
Sex, male	2.02 (1.15–3.57)	.015[c]
Body mass index, kg/m^2	1.08 (1.01–1.17)	.030[c]
Current smokers, yes	1.18 (.64–2.15)	.601
Drinkers[a], yes	.54 (.31–.96)	.036[c]
Hypertriglyceridemia, yes	.85 (.47–1.52)	.574
Hypo-HDL-cholesterolemia, yes	1.13 (.52–2.44)	.764
Hemoglobin A1c, %	1.23 (.52–2.92)	.634
ALDH2 G/A + A/A genotype	1.62 (1.04–2.52)	.032[c]

Data were calculated by logistic regression model analysis.
LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALDH, aldehyde dehydrogenase.
[a] Drinkers were defined as individuals who consumed 1 to 3 go/day on average.
[b] $P\leq.01$.
[c] $P\leq.05$.

Table 3. Multivariate-adjusted logistic regression analysis for hyper-LDL-cholesterolemia by genotype: the subgroup analysis of non-drinkers and drinkers

	Odds ratio (95% CI)	*P*
Non-drinkers ($n=288$)		
Age, years	1.07 (1.04–1.11)	<.001[b]
Sex, male	2.30 (1.21–4.37)	.011[c]
Body mass index, kg/m^2	1.06 (.97–1.15)	.184
Current smokers, yes	.84 (.38–1.84)	.659
Hypertriglyceridemia, yes	.69 (.34–1.38)	.292
Hypo-HDL-cholesterolemia, yes	.98 (.43–2.27)	.969
Hemoglobin A1c, %	.93 (.36–2.44)	.886
ALDH2 G/A + A/A genotype	1.70 (1.02–2.84)	.042[c]
Drinkers ($n=95$)[a]		
Age, years	1.00 (.93–1.09)	.929
Sex, male	2.93 (.52–16.37)	.222
Body mass index, kg/m^2	1.16 (.97–1.38)	.108
Current smokers, yes	1.81 (.67–4.93)	.246
Hypertriglyceridemia, yes	1.40 (.45–4.39)	.561
Hypo-HDL-cholesterolemia, yes	.32 (.04–3.48)	.351
Hemoglobin A1c, %	4.91 (.26–91.54)	.286
ALDH2 G/A + A/A genotype	1.56 (.60–4.07)	.359

Data were calculated by logistic regression model analysis.
LDL, low-density lipoprotein; HDL, high-density lipoprotein; ALDH, aldehyde dehydrogenase.
[a] Drinkers were defined as individuals who consumed 1 to 3 go/day on average.
[b] $P\leq.01$.
[c] $P\leq.05$.

Our study revealed a significantly lower HDL-C level in participants with the G/A genotype than in those with the G/G genotype, and a significantly higher prevalence of hyper-LDL-cholesterolemia in participants with the G/A genotype than those with the G/G genotype in a general Japanese population.

were not significantly associated with hypo-HDL-cholesterolemia. Although the analyses on hypo-HDL-cholesterolemia were performed in subgroups of non-drinkers or drinkers, no significant results related to the genotypes were observed.

DISCUSSION

Our study revealed a significantly lower HDL-C level in participants with the G/A genotype than in those with the G/G genotype, and a significantly higher prevalence of hyper-LDL-cholesterolemia in participants with the G/A genotype than those with the G/G genotype in a general Japanese population. It is valuable to note that the ALDH2 gene G/A polymorphism may differentially influence the lipid/lipoprotein profile, such as the HDL-C and LDL-C, which are both recognized as cardiovascular risk factors. Our study result of the association between the polymorphism and HDL-C was in agreement with earlier studies.[6,8,9] Although the association of the polymorphism with hypo-HDL-cholesterolemia was not evident from our study, this might be partly due to the different study methods and populations that were examined (eg, low prevalence of hypo-HDL-cholesterolemia) compared to the previous studies.[6,8,9]

Of note, our study result showing the significant increase in the presence of hyper-LDL-cholesterolemia in participants with the G/A + A/A genotype is the first description of such a finding. The detailed mechanisms underlying the current results remain unknown, while the ALDH2 gene G/A polymorphism has been indicated to have fundamental physiological roles in relation to lipid/lipoprotein metabolism, where the association is not always dependent of alcohol consumption (this independence was also observed in our study).[5,6,9] The fact that participants with the A allele showed slower metabolism of acetaldehyde may be related to the metabolic turnover of the other systems including lipids/lipoproteins, for example, leading to the delayed catabolism of apolipoprotein B-containing particles (accumulated LDL in the circulation) and a low transport rate of apolipoprotein A-I (relatively less HDL in the circulation).[11,12] In nature, various types of aldehydes exist in the human body, and ALDH2 is involved in the metabolism of not only alcohol-induced aldehydes but also aldehydes to various metabolites (ie, lipids, amino-acids).[13] Recently, in addition to lipid transport, an anti-cytotoxic detoxification function has been proposed as a function of lipoprotein particles.[14] Thus, our study result showing a higher presence of hyper-LDL-cholesterolemia in participants with the G/A + A/A genotype suggest that an increase in LDL particles may occur as a compensatory response to the disturbed acetaldehyde metabolism in the A allele carriers, although the evidence for this hypothesis is insufficient at present.

Furthermore, it is interesting to note that the association between hyper-LDL-cholesterolemia and carrying the G/A + A/A genotype appeared to be more clearly seen in non-drinkers than in drinkers (although the subgroup analysis of drinkers was performed on a smaller *n* relative to that of non-drinkers). These findings suggest that alcohol drinking habits might modulate the polymorphic effects on lipid/lipoprotein. Indeed, it has been demonstrated that the influence of the G/A polymorphism on the lipid profile could generally be seen clearly in non- or light-drinkers.[6,7,9] These issues should be investigated in greater detail in future studies.

This study had additional potential limitations. Its cross-sectional design did not completely allow us to draw conclusions about the cause-and-effect association between the relevant variables. There were few participants with the A/A genotype, and few females were smokers and drinkers. The detailed information about alcohol drinking habits (eg, alcohol types, exact amounts, exposure duration) was also lacking.

In summary, our study showed a significantly higher presence of hyper-LDL-cholesterolemia in participants with the G/A + A/A genotype of ALDH2 gene polymorphism. The observed phenomenon that the ALDH2 gene G/A polymorphism may affect the lipid/lipoprotein profile therefore requires more investigation.

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