Confirmation of ALDH2 as a Major Locus of Drinking Behavior and of Its Variants Regulating Multiple Metabolic Phenotypes in a Japanese Population

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Background: Normative alcohol use (or drinking behavior) influences the risk of cardiovascular disease in a multi-faceted manner. To identify susceptibility gene variants for drinking behavior, a 2-staged genome-wide association study was performed in a Japanese population.

Methods and Results: In the stage-1 scan, 733 cases and 729 controls were genotyped with 456,827 SNP markers. The associated loci without redundancy of linkage disequilibrium were further examined in the stage-2 general population panel comprising 2,794 drinkers (≥once per week), 1,521 chance drinkers (<once per week), and 1,351 non-drinkers. Along with genome-wide exploration, we aimed to replicate the trait association of a candidate gene SNP previously reported (rs12299984 in ADH1B). A cluster of 12 SNPs on 12q24 were found to significantly (P<5×10^-6) associate with drinking behavior in stage 1, among which rs671 (a Glu-to-Lys substitution at position 504) in the ALDH2 gene showed the strongest association (odds ratio (OR)=0.16, P=3.6×10^-211 in the joint analysis). The association was also replicated for rs1229984 (OR=1.20, P<3.6×10^-211). Furthermore, ALDH2 504Lys was associated with several metabolic traits, eg, lower levels of high-density lipoprotein cholesterol and liver enzymes—AST, ALT, and γGTP—by interacting with alcohol intake.

Conclusions: Our results confirm ALDH2 as a major locus regulating drinking behavior in the Japanese, indicating that the ALDH2 504Lys variant exerts pleiotropic effects on risk factors of cardiovascular disease among drinkers. (Circ J 2011; 75: 911–918)

Key Words: Alcohol; ALDH2; Asians; Genetic susceptibility; Genome-wide association study

Alcohol consumption, a lifestyle factor prevalent globally, has been suggested to be relevant to atherosclerotic risk factors, such as hypertension, obesity, dyslipidemia, and diabetes.1 Epidemiological studies have demonstrated that moderate alcohol intake lowers the risk of cardiovascular disease in a multi-faceted manner;2,3 for example, mild to moderate alcohol consumption has a favorable influence on high-density lipoprotein cholesterol (HDL-C) level4 and glucose metabolism,5 whereas it causes hypertension6 and hyper-triglyceridemia,6 on the other hand.

Both environmental and genetic factors are assumed to contribute to inter-individual differences in alcohol consumption.7 Here, alcohol drinking behavior can be broadly dichotomized in alcoholism (or alcohol dependence) and normative alcohol use. As alcohol dependence constitutes a substantial health and economic burden, much effort has been directed to clarify its pathogenesis. In this line, a genome-wide association (GWA) study has been recently performed for alcohol dependence besides test of candidate genes (eg, those involved in GABAergic function) in populations of European
Methods

Study Populations
We first performed GWA scan for alcohol drinking behavior using the genotype data on 1,462 Japanese samples (Table 1), which are part of our ongoing GWA study of cardiometabolic disorders among the Japanese as previously reported. Then, we examined the association signals in a general population, known as the Amagasaki Study, as described elsewhere. Briefly, to investigate lifestyle factors and genetic susceptibility to cardiovascular disease and its risk factor traits, we enrolled individuals who sought medical assessment from September 2002 to August 2003 at the Amagasaki Health Medical Foundation. The participants were included if they were over 18 years of age and had full clinical examination data, along with a completed questionnaire on their lifestyle. Among those enrolled in this prospective cohort study, 5,666 subjects (3,384 men/2,282 women) were used for the present association study.

Drinking behavior was assessed on the basis of the lifestyle questionnaire self-administered by the subjects. Alcohol consumption was classified into 2 categories: ever-drinkers (ie, ex-drinkers and current drinkers) and non-drinkers. Also, according to the current status and frequency of drinking, the subjects in the Amagasaki Study panel were categorized into 3 groups: non-drinker (n=1,351), chance drinker (less than once in a week, n=1,521) and drinker (equal to or more than once in a week, n=2,794). Since no strict distinction could be made by definition between chance drinkers and (habitual) drinkers, we arbitrarily defined habitual drinkers as above-mentioned in the present study, according to the national survey conducted in 2003 (http://www.e-healthnet.mhlw.go.jp/ (in Japanese, accessed on 9 Feb 2011). For chance drinkers and

From a public health viewpoint, however, we should give an equivalent degree of attention to normative alcohol use. Ethanol acts in areas of the brain and on signaling cascades that are evolutionarily conserved among mammals. It has been shown in rodents that low level of responses to alcohol, which appear to increase the risk for lifetime alcohol dependence in humans, are genetically determined in part. Rats selectively bred for alcohol preference and non-preference have been used for studying the behavioral and molecular basis of alcohol drinking. Biological mechanisms that are assumed to serve to promote and maintain a high alcohol drinking behavior are: (1) increased potency of low-dose alcohol as a reinforcer; (2) weaker aversion to the pharmacological effects of moderate/high doses of alcohol; and (3) rapid induction of tolerance to the aversive effects of alcohol with repeated bouts of voluntary alcohol drinking.

Apart from several candidate genes involved in ethanol and acetaldehyde metabolism, genetic susceptibility to normative alcohol use has not been widely explored. Therefore, in the present study, we performed a GWA study to identify susceptibility loci for alcohol drinking behavior in a Japanese population, followed by examination of their relevance to metabolic trait levels.

Table 1. Clinical Characteristics of Study Participants

<table>
<thead>
<tr>
<th></th>
<th>GWA study panel</th>
<th>Amagasaki Study panel</th>
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<tbody>
<tr>
<td></td>
<td>Drinker</td>
<td>Chance drinker</td>
</tr>
<tr>
<td>Number of subjects (M/F)</td>
<td>830/632</td>
<td>2,206/588</td>
</tr>
<tr>
<td>Age, year</td>
<td>66.3±8.0</td>
<td>49.8±11.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.5±3.3</td>
<td>23.2±2.9</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>NA</td>
<td>25.4±12.5</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>24.4±22.1</td>
<td>26.2±19.5</td>
</tr>
<tr>
<td>γGTP, IU/L</td>
<td>34.6±63.1</td>
<td>38.0±47.6</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>122.3±71.4</td>
<td>123.1±100.5</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>122.4±31.4</td>
<td>120.0±31.2</td>
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<tr>
<td>HDL-C, mg/dl</td>
<td>60.1±16.8</td>
<td>62.9±17.9</td>
</tr>
<tr>
<td>% of dyslipidemia treatment</td>
<td>57.5</td>
<td>2.7</td>
</tr>
<tr>
<td>% of gout treatment</td>
<td>6.1</td>
<td>2.3</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>5.38±0.62</td>
<td>5.5±1.1</td>
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<tr>
<td>% of diabetes treatment</td>
<td>30.8</td>
<td>6.5</td>
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<tr>
<td>SBP, mmHg</td>
<td>133.5±19.8</td>
<td>127.8±17.3</td>
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<tr>
<td>DBP, mmHg</td>
<td>76.6±11.9</td>
<td>78.4±10.8</td>
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<tr>
<td>% of hypertension treatment</td>
<td>47.8</td>
<td>11.8</td>
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<tr>
<td>Smoking behavior</td>
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<tr>
<td>Non smoker, %</td>
<td>55.8</td>
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<tr>
<td>Past smoker, %</td>
<td>11.6</td>
<td>13.9</td>
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<tr>
<td>Current smoker, %</td>
<td>32.7</td>
<td>44.0</td>
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<tr>
<td>ALDH2 rs671 genotype</td>
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<tr>
<td>%GG/%AG/%AA</td>
<td>56.0/37.6/6.4</td>
<td>72.7/27.1/0.2</td>
</tr>
</tbody>
</table>

Values are means±SD unless otherwise indicated.
In the Amagasaki Study panel, drinkers are defined as those who take alcoholic beverages ≥once a week, and chance drinkers are those who take alcoholic beverages <once a week. GWA, genome-wide association; BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.
drinkers, alcohol intake was self-reported as the usual amounts that were denoted in terms of servings of sake (1 gou (180 ml) of Japanese rice wine is considered equal to 22 g of ethanol). In addition, for part of drinkers (n=1,741), more detailed information was available on the type of alcoholic beverages consumed, the weekly frequency of alcohol consumption, and the usual amount of alcohol consumed per day; 1 gou of sake was defined as equivalent to 500 ml of beer, 2/3 gou of shochu, 2 single whiskeys, or 240 ml of wine. Based on the type and amount of beverage consumed, we calculated the total units (in gou) of ethanol for the weekly intake as a measure of alcohol intake in the quantitative analysis (Figure S1). The blood samples were collected during a visit to measure glucose, lipid, liver enzyme, and uric acid concentrations using standard techniques. Blood pressure was measured using an automatic sphygmomanometer in the sitting position after at least 5-min of rest. Smoking habits were classified into 3 categories: current smokers, past-smokers, and non-smokers.

All participants from these different studies provided written informed consent, and the local ethics committees approved the protocols.

SNP Genotyping and Quality Control
In the GWA scan, genotyping was performed with the Infinium HumanHap550 BeadArray (Illumina), which interrogated 555,352 SNPs (Data S1). Data cleaning and analysis were performed using PLINK software as described elsewhere. Population stratification was checked by multidimensional scaling analysis of the pair-wise distance between samples, measured over all SNPs (Data S1). The lambda value for the genomic control was 1.01, indicating the absence of systematic confounding, such as population stratification, in the GWA study panel (Data S1).

After the GWA scan, 2 additional SNPs, rs671 in ALDH2 and rs1229984 in ADH1B, were chosen for genotyping with the TaqMan assay (Applied Biosystems) as previously described in the GWA study (stage 1) panel. Here, rs671 and rs1229984 were added to the GWA scan because of their positional (rs671 on 1q24) and physiological (ALDH2 and ADH1B) candidacy. Although we had tested these 2 SNPs as part of 10 candidate SNP loci with relation to drinking behavior and the sensitivity to pressor effects of alcohol in our previous candidate gene approach, we re-evaluated them via genome-wide exploration and newly examined the pleotropic effects on metabolic traits (in the Amagasaki Study panel) using the refined (or extended) clinical/epidemiological data in the present study. Beside the 2 SNPs, 2 SNPs—rs10774610 and rs1725236—showing significant (P<5×10^-8) or suggestive (P<5×10^-7) evidence of association in the GWA scan were taken forward to replication analysis in the Amagasaki Study (stage 2) panel. The genotype distribution of all tested SNPs was in Hardy-Weinberg equilibrium (P>0.001). We obtained successful genotyping call rates of >99% for the whole characterized sample.

Statistical Analysis
SNP Association Analysis A joint analysis strategy, a staged design in which part of the available samples are genotyped on a large number of SNP markers in stage 1, and part of these markers are later followed up by genotyping them on the remaining samples in stage 2, was adopted in the present study. The SNPs were tested for the association with alcohol drinking behavior (ever-drinkers vs. non-drinkers) in the GWA study panel (stage 1: 733 cases and 729 controls) and in the combined panel (stage 1+2: 4,780 cases and 1,940
controls) by logistic regression analysis after adjustment for sex, age (GWA study and Amagasaki Study panels) and enrollment site (GWA study panel only). To combine the association results from the 2 panels, we used the inverse variance method (Table S1).

Positive Selection in the 12q24 Region Near ALDH2
While the present GWA study identified strong associations at several SNP loci in a >0.8-Mb interval on 12q24, they became no more significant after adjustment for rs671. Since a previous European GWA study on hematological traits claimed significant evidence for signatures of natural selection in the corresponding region on 12q24, we hypothesized that a long-range, evolutionarily derived haplotype, upon which effect alleles (A of rs671 with effect toward moderation of alcohol intake) could lie, arose from a positive selection in the Japanese. To test this hypothesis, we performed haplotype-based tests in the 12q24 region near ALDH2.

Effect Estimates of rs671 Genotype on Metabolic Traits
We performed multiple regression analysis to test the effects of ALDH2 rs671 genotype (AG vs. GG) on metabolic traits with adjustment for drinking status, sex and smoking habits as covariates (Table S2). Further, we evaluated the effects of ALDH2 rs671 genotype on metabolic traits in 2 ways: by treating drinkers as a category (Table 2); and by quantitative adjustment for weekly alcohol intake among drinkers (Table 3). To adjust for skewness in the distribution, AST, ALT, γGTP, triglycerides and HDL-C were log-transformed before regression analysis; the effect size estimates were then re-transformed to the original units.

Results
Characteristics of participants in the present study are summarized in Table 1. In the general population (Amagasaki Study) panel, there were various differences among 3 groups classified by drinking behavior. Chance drinkers were significantly (P<0.05) younger than both drinkers and non-drinkers. Concentrations of serum liver enzymes (AST, ALT, γGTP) and triglyceride were significantly higher in drinkers than in chance drinkers and non-drinkers. However, low-density lipoprotein cholesterol (LDL-C) levels were significantly lower in both drinkers and chance drinkers than in non-drinkers. Despite some differences in the ratio of people under treatment, concentrations of serum uric acid, plasma glucose, and blood pressure tended to be higher in drinkers as compared to those in non-drinkers. Apart from such inter-group differences in objective variables, smoking behavior largely differed among the 3 groups, partly due to the increased ratio of men to women in drinkers.

In the genome-wide exploration (stage 1), there was a...
single prominent peak on 12q24 (Figure 1); the strongest evidence of association with drinking behavior was identified for rs671 in ALDH2 (odds ratio (OR) = 0.31, P = 1.8 × 10^{-30}) among significant association signals (a total 12 SNPs showing P < 5 × 10^{-8}), which all clustered in the 12q24 region (Figure 2 and Table S3). With the genotype data adjusted for rs671, most of the association signals became no more significant apart from 2 SNPs, rs10774610 and rs11065774 (r^2 = 0.97 between the SNPs), at which a borderline (P < 0.05) level of association remained. In the joint analysis (stage 1 + 2), these associations were proven not to be independent of rs671 (Table S1). Besides, although a SNP, rs1725236, showed suggestive (P < 5 × 10^{-7}) association in stage-1 GWA scan, we could not verify it in the joint analysis. Instead, we could replicate a nominally significant association (OR = 1.20, P = 3.6 × 10^{-4}) in the joint analysis at rs1229984 in ADH1B, a candidate gene SNP previously reported (Table S1).

On 12q24, rs671 was found to be in strong linkage disequilibrium with rs3782886 in BRAP (r^2 = 0.87). These 2 SNPs and 6 other SNPs (rs11066001, rs11066015, rs46469776, rs11066132, rs2074356, and rs11066280) are polymorphic only in East Asians and they constituted the evolutionarily derived haplotype from the HapMap data. A long-range haplotype (>0.7 Mb estimated from these 8 SNPs) was hypothesized to have arisen from a positive selection specific to East Asians; this was supported by the slower decreases in extended haplotype homozygosity for the derived alleles than the ancestral alleles in the 12q24 region (Data S1 and Figure S2). Thereafter, we focused on rs671 because (1) it had the strongest association signal among the SNPs tested in stage-1 GWA scan, (2) the association of other individual SNPs did not remain significant when conditioning on rs671, and (3) there had been substantial evidence supporting the functional importance of rs671 (regarding the enzyme activity of ALDH2) in ethanol metabolism.

We then verified genetic impacts of rs671 on drinking behavior using Amagasaki Study samples in 2 analytical procedures, ie, categorical (or drinking status) and quantitative (in tertiles) classifications. According to drinking status—non-drinker, chance drinker, and drinker—genotype distribution of rs671 significantly differed in both sexes (eg, P = 2 × 10^{-29} for men and P = 2 × 10^{-22} for women, non-drinker vs. drinker) (Figure 3a). Those with AA homozygote of rs671 are non-drinkers (n = 325) except for 5 drinkers and 22 chance drinkers, according to the definition of drinking status in the present study. When drinkers (2,206 men and 588 women) were further categorized into 3 tertiles according to the amount of alcohol intake, clear stepwise increases in the G (alcohol tolerant-type)-allele frequency were observed in men (75% to 90%; eg, P = 1 × 10^{-13}, 1st vs. 3rd tertile) and women (85% to 94%; eg, P = 0.018, 1st vs. 3rd tertile) (Figure 3b). Also, such a correlation between allele/genotype of rs671 and the amount of alcohol intake could be detected when the drinkers were more quantitatively grouped into 5 strata (Figure S3).

In the context of gene—environment interaction, we investigated genetic effects of rs671 for a series of metabolic traits in 2 ways. First, when we treated drinkers as a single category, there were significant inter-genotype (GG vs. AG) differences in concentrations of serum liver enzymes—AST, ALT, and γGTP—by interacting with alcohol intake (Table S2). Of note is the fact that, as compared to GG-homozygous non-drinkers (Table 2 and Figure S4), ALT concentrations were significantly (P < 0.05) increased among GG-homozygous drinkers and significantly (P = 4.5 × 10^{-7}) decreased among AG-homozygous drinkers; diastolic blood pressure was significantly (P = 5.9 × 10^{-4}) increased among GG-homozygous drinkers but did not significantly change among AG-heterozygous drinkers. There were no significant inter-genotype (GG vs. AG) differences in BMI despite its potential interaction with alcohol intake (Table 2 and Table S2). Next, when we focused on drinkers to consider the effect of individual alcohol intake, inter-genotype (GG vs. AG) differences were still significant.

Figure 2. Plots of alcohol drinking behavior association in a 12q24 region near the ALDH2 locus. All genotyped SNPs in the current Japanese GWA scan are plotted with their −log_{10}(P values) for alcohol drinking behavior against chromosome positions (in Mb). SNPs attaining genome-wide significance (P < 5 × 10^{-8}) are colored in red.
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(\(P = 2.7 \times 10^{-4}\) to \(5.5 \times 10^{-13}\) after adjustment for alcohol intake) in concentrations of serum liver enzymes and also marginally significant \((P<0.05)\) in levels of serum HDL-C and plasma glucose (Table 3).

**Discussion**

The present study has confirmed ALDH2 as a major locus regulating drinking behavior in the Japanese population. This is the first GWA study published to date, which investigates genetic susceptibility to normative alcohol use. Although it did not attain statistical significance in the exploration stage, the association was also replicated for a candidate gene SNP, rs1229984 (in \(ADH1B\)), as previously reported.\[11,24,25\] This indicates the presence of susceptibility genes other than ALDH2 with relatively modest effects on drinking behavior. Important findings in our study came from the systematic examination of the association of ALDH2 with cardiovascular risk factors related to alcohol intake. Notably, our results have revealed that rs671 (in \(ALDH2\)) exerts substantial genetic impacts not only on drinking behavior but also on several alcohol-related metabolic traits including HDL-C and liver enzymes, at least in part, independently of the amount of alcohol intake among drinkers.

A number of previous studies have reported the relevance of \(ALDH2\) to the risk of developing alcoholism in East Asians.\[27,28\] Flushing and discomfort, such as headache and nausea, occur in many individuals of East Asian ancestry after drinking even small amounts of ethanol. This has been known to be related to a functional variant in the \(ALDH2\) gene (\(ALDH2\) 504Lys), a substitution of the Glu at codon position 504 with Lys,\[29\] which corresponds to the A-allele of rs671. This variant allele acts as a dominant negative with the heterozygote having considerably reduced enzyme activity and the homozygote having no activity. The major metabolic pathway for ethanol is degradation by ADH (alcohol dehydrogenase) enzymes to an intermediate metabolite, acetaldehyde, followed by its degradation to acetate by ALDH (aldehyde dehydrogenase) enzymes. There are 2 major ALDH isozymes in the liver: cytosolic ALDH1 and mitochondrial ALDH2. The reduced activity of ALDH2 can lead to increase in the transient concentrations of the toxic acetaldehyde after drinking, thereby protecting the individuals with the \(ALDH2\) 504Lys variant(s) from heavy drinking.

Besides the identification of \(ALDH2\) 504Lys as a major genetic determinant of drinking behavior in the Japanese via genome-wide exploration, the present study has provided insights into metabolic functions of \(ALDH2\) 504Lys among the variant carriers (principally AG heterozygote of rs671) of drinkers. In epidemiological studies, most of the apparent
benefit of moderate alcohol consumption on the risk of coronary artery disease (or myocardial infarction) has been attributed to an increase in HDL-C levels.\textsuperscript{2,3,6,31} In accordance with the previous study by Nakamura et al,\textsuperscript{32} HDL-C concentrations were significantly lower in the AG heterozygotes than the wild-type form homozygotes (GG) among drinkers (Table 3); this association was more prominent in heavier drinkers ($\beta=-3.92$ mg/dl, AG vs. GG in the upper tertile; Table S4) and in men (Table S5). Since female drinkers tend to have a moderate amount of alcohol consumption (Figure S1), the observed sexual dimorphism could be explained by a dose-dependent interaction between ALDH2 rs671 and HDL-C concentrations. Another Japanese study\textsuperscript{33} reported the association between ALDH2 504Lys and low HDL-C concentrations among non-drinkers; while there was the same direction of association ($\beta=-1.36$ mg/dl, AG vs. GG), the estimated effects of ALDH2 rs671 genotype for HDL-C concentrations did not reach a nominal significance level in the present study (Table 2 and Table S6). In contrast to HDL-C levels, LDL-C levels were significantly decreased among drinkers as compared to non-drinkers in the general population panel, in accordance with the previous report in the Japanese.\textsuperscript{32} That is, LDL-C levels were significantly higher in those with AG heterozygote than those with GG homozygote only among chance drinkers ($\beta=4.01$ mg/dl, $P=0.012$; Table S6), whereas this genotype–trait association was less prominent among drinkers (Tables 2,3). There were no significant differences in triglyceride concentrations between the rs671 genotype classes. Thus, despite the relatively modest strength of association, the ALDH2 504Lys variant confers overall genetic effects on lipid profile in the direction for atherogenesis; ie, a decrease in HDL-C levels and an increase in LDL-C levels. This may account for, at least in part, a potential biological link between ALDH2 and cardiac ischemia as reported by Chen et al,\textsuperscript{34} who identified ALDH2 as an enzyme whose activation correlated with reduced ischemic heart damage in rodent models.

It has to be noted that, although statistical significance appeared to be borderline, blood pressure was lower in those with AG heterozygote than those with GG homozygote among chance drinkers ($\beta=-1.70$ mmHg for systolic blood pressure, AG vs. GG ($P=0.018$; Table S6), in accordance with our previous report.\textsuperscript{11} Among non-drinkers, or drinkers stratified by the amount of alcohol intake, the genetic effect on blood pressure was not statistically significant (Tables S4, S6). Our data cannot provide sufficient evidence supporting the notion that this locus differently exerts pressor effects between heavy drinkers and non-heavy drinkers (data not shown). Moreover, we found that concentrations of serum liver enzymes (in particular, ALT) were significantly lower in those with AG heterozygote than those with GG homozygote among drinkers (Tables 2,3). This association remains highly significant among drinkers after adjustment for alcohol intake (Table 3); it is highly significant ($P=3.2\times10^{-6}$ to $4.3\times10^{-14}$) for all 3 parameters in men and significant ($P=9.8\times10^{-4}$) only for $\gamma$GTP in women (Table S5). The findings of sexual dimorphism could be related to differences in the average amount of alcohol consumption between men and women (Figure S1). These ‘protective’ effects on liver tissue (or fatty liver disease) are in good agreement with a previous study in Japanese male workers\textsuperscript{35} and the study of Aldh2 knockout mice.\textsuperscript{36} Taken together, ALDH2 504Lys might exert some genetic effects on metabolic traits other than lipids in the direction against atherogenesis. Further investigation is warranted to clarify such pleiotropic or bi-directional functions of ALDH2 504Lys among drinkers.

Several studies including ours (Figure S2)\textsuperscript{37,38} have supported the hypothesis that positive selection has operated on ALDH2 504Lys; however, conclusive results have not been obtained thus far, partly due to the current methodological limitations.\textsuperscript{39} The restricted geographic distribution and high frequency of the physiologically important ALDH2 504Lys allele in East Asia has been of longstanding interest to human population geneticists. Additional information on genomic structure will be available from the ongoing 1,000 genomes project (http://www.1000genomes.org/; accessed on 9 Feb 2011) and helps to resolve this debated issue.

There are some limitations in the present study with regard to study design and data interpretation. We attempted to explore genetic factors influencing alcohol drinking behavior in normative alcohol use. Although we used the total units of ethanol for the weekly intake as a measure of alcohol intake in the quantitative analysis, this indicator of drinking behavior seems to be rather subjective, eg, the frequency of drinking on weekdays can be largely influenced by factors other than alcohol tolerance. In the general population, drinking behavior (or alcohol preference) is supposed to be defined by a combination of factors including genetic and environmental factors. That is, besides age and sex, a number of socio-economic factors such as occupation, place of residence, income, and lifestyle, the information of which is unavailable in the present study, exert some effects on the individual drinking behavior. Further, comprehensive multivariate analysis including the non-genetic factors as covariates is warranted to evaluate the relative contribution of alcohol intolerance, which is substantially defined by the ALDH2 504Lys allele, to the inter-individual variation in drinking behavior.

In summary, the present GWAS study has identified the ALDH2 504Lys allele and haplotype as a major determinant of genetic susceptibility to normative alcohol use in the Japanese. People with ALDH2 504Lys homozygote cannot tolerate the aversive effects of alcohol in principle. Those with heterozygote genotype are also less likely to become drinkers; once they regularly drink some amounts of alcohol, bi-directional influences of the variant on atherogenesis will be brought about with relation to cardiovascular disease.

**Acknowledgments**

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**References**


Supplemental Files

Data S1. Supplemental Note

Table S1. Loci Identified or Tested for Association With Drinking Behavior in the Japanese Population

Table S2. Estimated Effects of ALDH2 rs671 Genotype on Metabolic Traits by Regression Analysis With Adjustment for Drinking Status and Sex as Covariates

Table S3. Association With Drinking Behavior in the 12q24 Region

Table S4. Estimated Effects of ALDH2 rs671 Genotype on Metabolic Traits by Regression Analysis in Drinkers Stratified by Tertiles of Weekly Alcohol Intake

Table S5. Estimated Effects of ALDH2 rs671 Genotype on Metabolic Traits by Regression Analysis With and Without Adjustment for Weekly Alcohol Intake After Stratification by Sex

Table S6. Estimated Effects of ALDH2 rs671 Genotype on Metabolic Traits Among Chance Drinkers and Non-Drinkers

Figure S1. Histograms of weekly alcohol intake among drinkers: total sample (n=1,741) in the top left, men (n=1,311) in the top right, and women (n=430) in the bottom.

Figure S2. Suggestive evidence for positive selection in a 12q24 region near the ALDH2 locus.

Figure S3. Allele frequency (a) and genotype (b) distributions of ALDH2 rs671 according to the amount of alcohol intake among drinkers.

Figure S4. Estimated changes in metabolic traits according to rs671 genotype (GG vs. AG) and drinking status.

Please find supplemental file(s); http://dx.doi.org/10.1253/circj.CJ-10-0774