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The causal effects of alcohol on lipoprotein subfraction and triglyceride levels using a Mendelian randomization analysis: The Nagahama study

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ABSTRACT

Background: Light-to-moderate alcohol consumption may increase circulating high-density lipoprotein cholesterol (HDL-C) levels and decrease low-density lipoprotein cholesterol (LDL-C) levels. However, the effect of alcohol on biologically important lipoprotein subfractions remains largely unknown. Here we aimed to clarify the effects of alcohol on lipoprotein subfractions using a Mendelian randomization analysis.

Methods: The study subjects consisted of 8364 general Japanese individuals. The rs671 polymorphism in aldehyde dehydrogenase 2 gene, a rate-controlling enzyme of alcohol metabolism, was used as an instrumental variable. Lipoprotein subfractions were measured by a homogeneous assay.

Results: The biologically active *1 allele of the *ALDH2* genotype was strongly associated with alcohol consumption in men ($p < 0.001$). In a regression analysis adjusted for possible covariates, the *1 allele was positively associated with HDL-C even in a sub-analysis for HDL subfractions (HDL2-C: $\beta = 0.082$, $p < 0.001$; HDL3-C: $\beta = 0.195$, $p < 0.001$). In contrast, the *1 allele was inversely associated with total LDL-C levels ($\beta = -0.049$, $p = 0.008$), while its association with large-buoyant LDL-C ($\beta = -0.124$, $p < 0.001$) and small-dense LDL-C ($\beta = 0.069$, $p < 0.001$) was opposite. Therefore, the ratio of small-dense LDL to large-buoyant LDL exhibited a linear increase with the number of *1 alleles carried ($\beta = 0.127$, $p < 0.001$). Furthermore, the *1 allele was inversely associated with triglyceride levels in an analysis adjusted for LDL subfractions ($\beta = -0.097$, $p < 0.001$), but not for the total LDL ($\beta = 0.014$, $p = 0.410$).

Conclusions: Alcohol may increase HDL-C levels irrespective of the particle size. Moreover, alcohol may decrease the total LDL-C, although the proportion of atherogenic small-dense LDL-C increased partially due to a potential inter-relationship with decreased triglyceride levels.

1. Introduction

It has been well documented that light-to-moderate alcohol consumption can reduce the risk of developing cardiovascular disease [1]. Although the mechanism for the favorable effect of alcohol consumption has not been fully elucidated, an improvement of the lipoprotein profiles, particularly increased levels of circulating high-density lipoprotein cholesterol (HDL-C) [2,3], is thought to provide a potential explanation. In addition to the HDL-C increasing effect of alcohol, previous epidemiological studies [3–5] (including our own [6]) have found that alcohol may also have a low-density lipoprotein cholesterol (LDL-C) lowering effect. Since the cardioprotective activity of HDL, as well as the atherogenic properties of LDL, may differ based on their respective subfractions

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[7–9], the effect of alcohol on the circulating levels of lipoprotein subfractions should be clarified to further understand the mechanisms behind the beneficial effects of alcohol. The effect of alcohol on triglyceride (TG) levels is another inconclusive aspect, with studies reporting both reduced [10,11] and increased [12] TG levels in alcohol drinkers.

One challenge in clarifying the effect of alcohol in an epidemiological study setting is the other confounding clinical factors that are potentially affected by alcohol. A Mendelian randomization analysis (MRA) is a method used to clarify the causality of the risk factor of interest for a given outcome by using a genotype robustly associated with the risk factor as an instrumental variable [13]. Given that genes are randomly assigned during meiosis, a genetic variant is fundamentally independent of typical confounding factors. Most East Asians, including Japanese, have the inactive allele (*2 allele) for the aldehyde dehydrogenase 2 (*ALDH2*) gene, a rate-controlling enzyme in ethanol metabolism. Therefore, individuals that are homozygotes for *2 alleles tend to be non-drinkers, while the daily alcohol consumption of homozygotes for the enzymatically active *1 allele are reported to be approximately double than that of heterozygotes [6,14–16]. A MRA using the *ALDH2* genotype in Japanese individuals is a convincing approach to clarify the effects of alcohol on the circulating levels of lipoprotein subfractions and triglycerides.

In the present study, to clarify the pleiotropic effect of alcohol, we assessed the causality between alcohol intake and the circulating levels of lipoprotein subfractions and triglycerides in a large general Japanese population by a MRA using the *ALDH2* genotype as an instrumental variable.

2. Materials and methods

2.1. Study participants

We analyzed a dataset of the Nagahama Prospective Cohort for Comprehensive Human Bioscience (the Nagahama Study). The study participants in this cohort were recruited from 2008 to 2010 from the general population of Nagahama City, a largely rural city consisting of 125,000 inhabitants located in central Japan. The inclusion criteria for this cohort consisted of the following: community residents aged from 30 to 74 years, living independently in the community, and without physical impairment or dysfunction. Among a total of 9804 participants, the remaining 9769 individuals after the exclusion of the following conditions: individuals that withdrew consent to participate in this study (n = 9), and that proved to have a different ethnic background by genetic analysis (n = 26).

The baseline measurements were performed at the time of recruitment. Of the 9769 potential participants, those meeting any of the following conditions were excluded from this study: pregnant women (n = 43), individuals undergoing insulin therapy (n = 25) or lipid-lowering medications (n = 1171), an unsuccessful measurement or extreme deviation of lipoprotein cholesterol subfractions (n = 25) or other clinical parameters required for this study (n = 107), and the unavailability of a *ADLH2* rs671 genotype (n = 34). We did not consider absorption disorders in this study population. A final total of the remaining 8364 participants were considered as this study population.

All study procedures were approved by the ethics committee of the Kyoto University Graduate School of Medicine and by the Nagahama Municipal Review Board. Written informed consent was obtained from all participants.

2.2. Basic clinical parameters

The basic clinical parameters used in this study were obtained from the baseline measurements. Circulating levels of triglyceride (Determiner C-TG, Kyowa Medex, Co., Ltd. Tokyo, Japan) and total cholesterol (Determiner C-TC) levels were measured using serum samples. The history of cardiovascular diseases (CVD), menopausal status, smoking habits, amount of alcohol consumed in a single sitting, and medication use were obtained using a structured, self-administered questionnaire. The level of alcohol consumption was described in Japanese traditional units (Go), in which 1 Go corresponds to 22 g of ethanol. A peripheral blood sample from a number of participants was collected under non-fasting (<5 h, n = 693) or near-fasting (5–11 h, n = 3831) conditions.

2.3. Measurement of lipoprotein subfractions

Circulating levels of lipoprotein subfractions were measured using a plasma sample stored at −80 °C. To directly measure HDL3-C, TG-rich lipoproteins and LDL were first digested using sphingomyelinase, and the cholesterol released from these lipoproteins was then eliminated using cholesterol esterase/oxidase and catalase. Cholesterol in HDL3 was then measured via a standard peroxidase method following enzymatic treatment with a polyoxyethylene styrenated phenyl ether derivative that specifically acts on HDL3 (HDL3-EX, Denka Seiken Tokyo, Japan) [17]. HDL2-C levels were then calculated by subtracting the HDL3-C from the total HDL-C measured using a commercially available assay kit (HDL-EX, Denka Seiken). A strong collinearity between the HDL subfraction levels measured using this method and those measured by an objective standard method via ultracentrifugation has been reported elsewhere [17].

Small dense LDL cholesterol (sdLDL-C) was measured using a standard cholesterol assay following enzymatic treatment with a polyoxyethylene benzylphenyl ether derivative for eliminating TG-rich lipoproteins and HDL, as well as a sphingomyelinase that specifically reacts with large buoyant LDL (lbLDL) (sdLDL-EX, Denka Seiken) [18]. The total LDL cholesterol was measured using a commercially available assay kit (LDL-EX (N), Denka Seiken). lbLDL-C levels were calculated by subtracting the sdLDL-C from the total LDL-C. The accuracy of the LDL subfraction levels measured using this method has been reported elsewhere [18].

2.4. DNA extraction and genotyping

Genomic DNA was extracted from the peripheral blood using a conventional phenol-chloroform method. The *ALDH2* rs671 genotype was analyzed using a series of BeadChip DNA arrays (Illumina, San Diego, CA, USA), or via a TaqMan probe assay (Applied Biosystems Co., Ltd., Foster City, CA, USA) using commercially available primers and probes purchased from the Assay-on-Demand system (C_11703892_10). The fluorescence of TaqMan-PCR products was measured using an ABI PRISM 7900HT sequence detector (Applied Biosystems). Alleles for G (Glu) and A (Lys) were considered as the *1 and *2 alleles, respectively.

2.5. Statistical analysis

Differences in the numerical variables were assessed using an analysis of variance, while the frequency differences were assessed using a chi-squared test. Associations of the *ALDH2* genotype with plasma markers were evaluated via a linear regression analysis with an additive genetic model (*1*1 = 2, *1*2 = 1, *2*2 = 0). Statistical analyses were performed using commercially available statistical software (JMP ver. 9.03; SAS Institute, Cary, NC, USA). Null