Abstract

Introduction
Health benefits of low-to-moderate alcohol consumption may operate through an improved lipid profile. A Mendelian randomization (MR) approach was used to examine whether alcohol consumption causally affects lipid levels.

Methods
This analysis involved 10,893 European Americans (EA) from the Atherosclerosis Risk in Communities (ARIC) study. Common and rare variants in alcohol dehydrogenase and acetaldehyde dehydrogenase genes were evaluated for MR assumptions. Five variants, residing in the ADH1B, ADH1C, and ADH4 genes, were selected as genetic instruments and were combined into an unweighted genetic score. Triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c) and its subfractions (HDL2-c and HDL3-c), low-density lipoprotein cholesterol (LDL-c), small dense LDL-c (sdLDL-c), apolipoprotein B (apoB), and lipoprotein (a) (Lp(a)) levels were analyzed.

Results
Alcohol consumption significantly increased HDL2-c and reduced TG, total cholesterol, LDL-c, sdLDL-c, and apoB levels. For each of these lipids a non-linear trend was observed. Compared to the first quartile of alcohol consumption, the third quartile had a 12.3% lower level of TG (p < 0.001), a 7.71 mg/dL lower level of total cholesterol (p = 0.007), a 10.3% higher level of HDL2-c (p = 0.007), a 6.87 mg/dL lower level of LDL-c (p = 0.012), a 7.4% lower level of sdLDL-c (p = 0.037), and a 3.5% lower level of apoB (p = 0.058, poverall = 0.022).
Conclusions
This study supports the causal role of regular low-to-moderate alcohol consumption in increasing HDL2-c, reducing TG, total cholesterol, and LDL-c, and provides evidence for the novel finding that low-to-moderate consumption of alcohol reduces apoB and sdLDL-c levels among EA. However, given the nonlinearity of the effect of alcohol consumption, even within the range of low-to-moderate drinking, increased consumption does not always result in a larger benefit.

Introduction
Low-to-moderate alcohol consumption has been associated with cardiovascular health benefits in observational [1–3] and experimental studies [4,5], but the mechanism is still unclear. Alcohol consumption may introduce a cardiovascular benefit by improving an individual’s lipid profile, including an effect on HDL-c levels, HDL particle concentration, and HDL-c subfractions [6,7]. The relationship between low-to-moderate alcohol use and LDL-c or TG is less clear, with studies reporting reduced LDL-c or TG levels [8–15], no effect [4], or a worsened blood lipid profile [5,13,16].

The observed association between alcohol use and lipids can be confounded by demographic, social and behavioral factors, as well as access to health care, and health-related conditions [17,18]. Mendelian randomization (MR) studies using instrumental variable (IV) analysis and genetic instruments can facilitate causal inference in observational studies by reducing the issues of residual confounding and reverse causation [19–27]. Using genetic variants that influence alcohol consumption may better capture the role of life-long alcohol use [28]. A limited number of MR studies have been conducted to evaluate the relationship between alcohol consumption and lipid levels, and the results have been largely inconclusive [2,28,29]. There is no previous MR study investigating the causal role of alcohol consumption on HDL-c subfractions, HDL2-c and HDL3-c. Evaluation of these subfractions may provide a more complete picture, as HDL is highly heterogeneous in terms of particle size, lipid component, and functionality and it is hypothesized that not all HDL subclasses have anti-atherogenic effects [30]. The relationship between alcohol consumption and sdLDL-c, apoB, and Lp(a) has also not been previously investigated. This MR study aims to investigate the causal link between low-to-moderate alcohol consumption and blood levels of TG, total cholesterol, HDL-c, HDL2-c, HDL3-c, LDL-c, sdLDL-c, apoB, and Lp(a) among European Americans (EAs).

Methods
This study involved 10,893 EAs from the Atherosclerosis Risk in Communities (ARIC) study, an ongoing prospective cohort in four communities in the US: Forsyth County, North Carolina; Washington County, Maryland; Minneapolis suburbs, Minnesota; and Jackson, Mississippi. A description of the ARIC study objectives, design, and procedures is provided elsewhere [31]. Briefly, ARIC participants received an extensive baseline examination (1987–1989), including collection of medical, social, and demographic data. Three follow-up examinations were performed at three-year intervals, and a fifth exam was conducted in 2011 to 2013. Participant follow-up also occurred annually, by telephone, to maintain contact and to assess health and vital status of the cohort. Individuals were categorized as EAs by self-report. The ARIC study has been approved by Institutional Review Boards (IRB) at all participating institutions:

Competing Interests: The authors have declared that no competing interests exist.
This study focused on self-reported alcohol consumption at two of the examinations, baseline and visit 4, during which the lipid levels included in this study were measured. Participants were interviewed in person using a dietary questionnaire, and they were asked if they currently or formerly drank alcoholic beverages. For current drinkers, information about the frequency and amount of wine, beer, or hard liquor consumption was collected. The amount of alcohol consumed in grams per week (g/wk) was calculated with the estimate that 4 oz wine was equal to 10.8 g, 12 oz beer was equal to 13.2 g, and 1.5 oz liquor was equal to 15.1 g ethanol. Alcohol consumption was recorded as 0 g/wk for current drinkers having less than one drink per week. Total alcohol consumption was analyzed as the natural log of (alcohol use in g/wk +1) given the skewed distribution. Current drinkers were further classified as infrequent drinkers if they had less than one drink per week, as low-to-moderate drinkers if they drank ≤ 210 g/wk for men and ≤ 105 g/wk for women, and were classified as heavy drinkers if they drank > 210 g/wk for men and > 105 g/wk for women [32–35].

TG, total cholesterol, HDL-c and its subfractions, LDL-c, and Lp(a) were measured from 12-hour fasting blood samples collected at baseline. Plasma total cholesterol [36] and TG [37] were measured by enzymatic methods, with the use of reagents supplied by Boehringer-Mannheim Biochemical, and were adapted for analysis in the Cobas-Bioanalyzer (Roche). HDL-c level was determined by measuring cholesterol in the supernate after plasma precipitation with MgCl₂ and dextran sulfate according to the method of Warnick et al [38]. LDL₃-c level was determined after reprecipitation of the total HDL-c supernate with different concentrations of MgCl₂ and dextran sulfate. LDL₂-c level was calculated by subtracting the HDL₃-c value from the value of total HDL-c [39]. LDL-c was calculated from the levels of total cholesterol, HDL-c, and TG by the Friedewald formula [40]. LDL-c was not determined in individuals with plasma TG levels >400 mg/dL [41]. Lp(a) was measured as total protein component (apolipoprotein A plus apoB) with a double-antibody ELISA technique for apolipoprotein A detection [42]. Since Lp(a) assay used in ARIC at baseline could be sensitive to apo(a) isoform size, we performed a correction, multiplying by a factor of 1.326, to match with a newer Lp(a) assay that is insensitive to apo(a) isoform size when calibrated with the International Federation of Clinical Chemistry proposed reference material in molar units [43]. SdLDL-c and apoB were measured from 12-hour fasting blood samples collected at visit 4. SdLDL-c was directly measured by a homogeneous assay method (sd-LDL-EX “Seiken”, Denka Seiken, Tokyo, Japan) on a Hitachi 917 automated chemistry analyzer [44]. ApoB was measured by an immunonephelometric assay using a BNII nephelometer (Siemens Healthcare Diagnostics, Deerfield, IL) [44]. Due to the skewed distribution of TG, HDL-c, HDL₂-c, sdLDL-c, apoB, and Lp(a), these measures were evaluated using the natural log transform.

In humans, alcohol is converted to acetaldehyde by alcohol dehydrogenases (ADH), and then to acetate by acetaldehyde dehydrogenases (ALDH) [45]. Variants in genes from the ADH and ALDH gene families are often associated with reduced drinking as they raise the blood level of acetaldehyde which causes uncomfortable symptoms such as “hangover”, nausea, and facial flush [46]. Therefore, genetic variation in the ADH and ALDH genes were the focus of this study. Previously published association studies, most of which involved European ancestry populations, identified 18 single nucleotide polymorphisms (SNPs) in ADH and ALDH genes (S1 Table) that were significantly associated with alcohol consumption [47–61]. These SNPs were evaluated for instrument selection by first identifying their availability in ARIC based on genotypes from the Infinium HumanExome BeadChip v1.0 (Illumina, Inc., San Diego, CA) [62] referred to as the “exome chip” hereafter, genotypes from the Affymetrix University of North Carolina at Chapel Hill IRB, Johns Hopkins University IRB, University of Minnesota IRB, and University of Mississippi Medical Center IRB. Study participants provided written informed consent at all study visits.
6.0 array [63], or those that were genotyped by TaqMan [64]. From ten SNPs available in ARIC, 6 SNPs in the ADH and ALDH genes passed stringent quality control procedures [62] (S1 Table).

Next, the 6 SNPs were evaluated for violation of IV assumptions. Since these assumptions are violated if the genetic instruments are in high linkage disequilibrium (LD) with loci associated with lipid levels [19, 23], the SNPs were examined for LD ($r^2 > 0.2$) with lipid-related loci identified in published genome wide association studies (GWAS) [65–76]. The SNPs were then evaluated using Pearson’s correlation coefficient for association ($r > 0.1$) with potentially genetically determined confounders of the alcohol consumption-lipid relationship. These confounders included smoking, body mass index (BMI), waist-to-hip ratio, and diabetes. To avoid redundancy, the selected SNPs were also evaluated for pair-wise LD ($r^2 > 0.7$), and only the SNP with the most functional impact (i.e., exonic, splicing), followed by the largest sample size, was kept. None of the 6 SNPs were correlated with potential confounders, nor were they in high LD with lipid-related loci, but rs1693482 was removed due to LD with another instrumental SNP ($r^2 = 0.96$) (S1 Table). A total of 5 SNPs (rs2066702, rs1693457, rs1789891, rs698, and rs1126671) met the IV assumptions (S1 Table).

The final genetic instruments were coded to ensure consistent effect direction of increasing alcohol consumption [77] and then were combined into an unweighted genetic risk score. The genetic risk score was further evaluated for correlation with lipid-related loci and potential confounders using the same criteria applied for the aforementioned SNP selection. The genetic risk score did not violate these MR assumptions, and was used to fit the IV regression models.

First we evaluated the regression between observed alcohol consumption categories and lipid levels to evaluate the linearity of the association. Two Stage Least Square (2SLS) IV regression, performed in Stata 12 [25], was used for causal inference and estimation of the causal effect size of alcohol consumption on lipid measures. Non-linear relationships between alcohol consumption and lipids were evaluated in the second stage of 2SLS using the predicted alcohol consumption categorized into quartiles and then fitted into the models with lipids. The significance of alcohol consumption was evaluated by both Wald $p$-values from the test comparing each quartile versus the first quartile and Wald $p$-values for overall significance of alcohol consumption. Population stratification was controlled for [23] using the first two genetic principal component scores calculated in Eigenstrat [78] based on genome-wide autosomal SNPs from the exome chip array. The IV regression models also controlled for sex and age to increase precision and reduce weak instrument bias [20]. With sdLDL-c and apoB measured at visit 4, alcohol consumption and age at visit 4 were included in the model. Similarly, with all other lipid outcomes measured at baseline, alcohol consumption and age at baseline were used. The IV regression diagnostics included a test of weak instrument bias using the F-value of the first stage regression, and an F-value greater than 10 was considered unbiased [20,21]. Because the effect of alcohol may have a J-shaped relationship with lipids and heavy drinking can be harmful [10, 12,79,80] sensitivity analyses excluding heavy drinkers were conducted to examine the effect of alcohol consumption within the low-to-moderate range. In addition, the relationship between alcohol consumption and lipids can be confounded or biased by reverse causation when individuals with adverse health conditions abstain from drinking. Although the MR approach is known to reduce the problems of confounding and reverse causation in observational data [19–27,81], we also conducted a sensitivity analysis excluding never drinkers, never and heavy drinkers, and never and former drinkers in order to further reduce the potential confounding or reverse causation.
### Results

Table 1 shows the characteristics of the ARIC EA individuals included in this study. Regular alcohol consumption was common, and 43.1% of individuals had one or more drink per week. Low-to-moderate current drinkers accounted for 34.4% and heavy drinkers only accounted for 8.8% of individuals.

The regression between observed alcohol consumption categories and lipid levels (Table 2) shows that increased alcohol consumption was associated with lower LDL-c and higher HDL-c, HDL2-c, and HDL3-c levels. A non-linear trend was observed for TG, total cholesterol, and sdLDL-c, and no significant association was observed with apoB and Lp(a). As a result of this observed non-linear relationship between alcohol consumption and the lipids evaluated as a part of this study, in the second stage of 2SLS the predicted alcohol consumption was categorized into quartiles and then fitted into the models with lipids.

Table 3 shows the 5 instrumental SNPs included in this MR study. The effect direction for alcohol consumption of all these 5 final instrumental SNPs was consistent with previous studies [48,52,55,58–60].

#### Table 1. Characteristics of 10,893 ARIC EAs.

| Characteristics          | n (%) or mean (SD)     |
|--------------------------|------------------------|
| Female                   | 5,784 (53.10%)         |
| Age (years)              | 54.3 (5.7)             |
| Alcohol consumption      |                        |
| grams/week               | 45.7 (93.9)            |
| Never Drinkers           | 1,965 (18.10%)         |
| Former Drinkers          | 1,848 (17.00%)         |
| Infrequent Drinkers*     | 2,369 (21.80%)         |
| Low-to-Moderate Current Drinkers* | 3,734 (34.36%) |
| Heavy Current Drinkers*  | 951 (8.75%)            |
| TG (mg/dL)               | 137.1 (90.7)           |
| Total cholesterol (mg/dL)| 214.5 (40.0)           |
| HDL-c (mg/dL)            | 50.5 (16.8)            |
| HDL2-c (mg/dL)           | 13.7 (8.6)             |
| HDL3-c (mg/dL)           | 36.8 (10.9)            |
| LDL-c (mg/dL)            | 137.6 (37.6)           |
| sdLDL-c (mg/dL)†         | 45.2 (21.0)            |
| apoB (mg/dL)†            | 100.6 (25.1)           |
| Lp(a) (mg/dL)†           | 8.2 (3.0–19.6)         |
| Smoking                  |                        |
| never smokers            | 4,386 (40.30%)         |
| former smokers           | 3,851 (35.40%)         |
| current smokers          | 2,649 (24.30%)         |
| Body mass index (kg/m²)  | 27.0 (4.9)             |
| Waist-to-hip ratio       | 0.9 (0.1)              |
| Diabetic                 | 955 (8.78%)            |

SD: standard deviation;
*Infrequent: < 1 drink/wk, Low-to-moderate: ≥ 1 drink & ≤ 210 g/wk for men and ≤ 105 g/wk for women, Heavy: ≥ 1 drink & > 210 g/wk for men and > 105 g/wk for women;
†measured at visit 4, lower sample sizes: N = 8,694 and 8,221 for sdLDL-c and apoB, respectively;
* median and interquartile range.

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The unweighted score created from the final five instrumental SNPs was associated with increased alcohol consumption at baseline ($\beta = 0.06$, $p < 0.001$) (S2 Table). This score explained approximately 0.1% variance of alcohol consumption (Table 4). Almost all 2SLS models using this score had first-stage F-values greater than 10, suggesting no weak instrument

| Lipids | Alcohol consumption categories                  | beta  | 95% CI   | p$^a$ |
|--------|-----------------------------------------------|-------|----------|-------|
| TG $^\text{¥}$ | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -0.08 | -0.11    | -0.05 | <0.001|
|        | Low-to-Moderate Current Drinkers              | -0.16 | -0.19    | -0.13 | <0.001|
|        | Heavy Current Drinkers                        | -0.13 | -0.17    | -0.09 | <0.001|
| Total cholesterol | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -2.75 | -4.99    | -0.51 | 0.02  |
|        | Low-to-Moderate Current Drinkers              | -0.73 | -3.03    | 1.57  | 0.53  |
|        | Heavy Current Drinkers                        | 4.05  | 0.73     | 7.37  | 0.02  |
| HDL-c $^\text{¥}$ | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | 0.03  | 0.01     | 0.04  | 0.001 |
|        | Low-to-Moderate Current Drinkers              | 0.14  | 0.12     | 0.15  | <0.001|
|        | Heavy Current Drinkers                        | 0.26  | 0.23     | 0.28  | <0.001|
| HDL2-c $^\text{¥}$ | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | 0.07  | 0.04     | 0.10  | <0.001|
|        | Low-to-Moderate Current Drinkers              | 0.17  | 0.14     | 0.20  | <0.001|
|        | Heavy Current Drinkers                        | 0.30  | 0.26     | 0.35  | <0.001|
| HDL3-c | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | 0.40  | -0.14    | 0.94  | 0.15  |
|        | Low-to-Moderate Current Drinkers              | 4.16  | 3.60     | 4.73  | <0.001|
|        | Heavy Current Drinkers                        | 8.70  | 7.84     | 9.55  | <0.001|
| LDL-c  | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -2.59 | -4.69    | -0.49 | 0.02  |
|        | Low-to-Moderate Current Drinkers              | -4.38 | -6.53    | -2.23 | <0.001|
|        | Heavy Current Drinkers                        | -7.48 | -10.70   | -4.25 | <0.001|
| sdLDL-c $^\text{¥a}$ | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -0.04 | -0.07    | -0.01 | 0.02  |
|        | Low-to-Moderate Current Drinkers              | -0.05 | -0.08    | -0.01 | 0.01  |
|        | Heavy Current Drinkers                        | 0.01  | -0.04    | 0.06  | 0.66  |
| apoB $^\text{¥a}$ | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -0.01 | -0.02    | 0.01  | 0.52  |
|        | Low-to-Moderate Current Drinkers              | -0.01 | -0.03    | 0.01  | 0.54  |
|        | Heavy Current Drinkers                        | -0.02 | -0.04    | 0.01  | 0.21  |
| Lp(a) $^\text{¥}$  | Never Drinkers*                              | 0.00  |          |       |
|        | Former/ Infrequent Drinkers                   | -0.03 | -0.10    | 0.03  | 0.34  |
|        | Low-to-Moderate Current Drinkers              | 0.01  | -0.06    | 0.08  | 0.72  |
|        | Heavy Current Drinkers                        | -0.06 | -0.16    | 0.03  | 0.19  |

*reference group,

$^\text{¥}$ In transformed,

$^\text{¥a}$ measured at visit 4,

$^\text{¥Wald}$ p-value comparing each alcohol consumption category with never drinkers.

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The unweighted score created from the final five instrumental SNPs was associated with increased alcohol consumption at baseline ($\beta = 0.06$, $p < 0.001$) (S2 Table). This score explained approximately 0.1% variance of alcohol consumption (Table 4). Almost all 2SLS models using this score had first-stage F-values greater than 10, suggesting no weak instrument
bias [20,21] except for sdLDL-c and apoB that were measured at visit 4 and therefore had reduced sample sizes (Table 4).

Table 4 shows significant causal relationships between alcohol consumption and TG, total cholesterol, HDL2-c, LDL-c, sdLDL-c, and apoB. Alcohol consumption increased HDL2-c and reduced TG, total cholesterol, LDL-c, sdLDL-c, and apoB levels. For all these lipids, the same non-linear trend was observed and the effect peaked at the third quartile and then reduced or stayed the same (only with sdLDL-c) at the fourth quartile. Compared to the first quartile, the third quartile of alcohol consumption had a 12.3% lower level of TG ($\beta$ in log scale ($\beta_{\log}$) = -0.13, 95%CI: -0.20, -0.07, $p < 0.001$), a 7.71 mg/dL lower level of total cholesterol ($\beta$ = -7.71, 95%CI: -13.26, -2.15, $p = 0.007$), a 10.3% higher level of HDL2-c ($\beta_{\log}$ = 0.10, 95%CI: 0.03, 0.17, $p = 0.007$), a 6.87 mg/dL lower level of LDL-c ($\beta$ = -6.87, 95%CI: -12.24, -1.50, $p = 0.012$), a 7.4% lower level of sdLDL-c ($\beta_{\log}$ = -0.08, 95%CI: -0.15, -0.005, $p = 0.037$), and a 3.5% lower level of apoB ($\beta_{\log}$ = -0.04, 95%CI: -0.07, 0.001, $p = 0.058$). With apoB, the second quartile had a slightly lower effect than the third quartile but it was significant ($p = 0.005$) due to a narrower 95%CI; and the test of overall effect of alcohol consumption was also significant ($p_{\text{overall}} = 0.022$).

The sensitivity analysis excluding heavy drinkers resulted in similar conclusions (Table 5). Specifically, TG, total cholesterol, HDL2-c, LDL-c, sdLDL-c, and apoB were still significant with similar patterns of effect. The lower $F$-values observed in Table 5 resulted from a smaller sample size due to the exclusion of the heavy drinkers.

The sensitivity analyses excluding never drinkers, never and heavy drinkers, and never and former drinkers (S3–S5 Tables) further confirms the significant causal role of alcohol consumptions on TG, total cholesterol, HDL2-c, LDL-c, sdLDL-c, and apoB.

**Discussion**

This study supports the causal role of regular low-to-moderate alcohol consumption in increasing HDL2-c, and reducing TG, total cholesterol and LDL-c, and provides evidence for the novel finding that low-to-moderate consumption of alcohol reduces apoB and sdLDL-c levels among EA. The association between alcohol consumption and increased HDL2-c levels was found in several observational studies [7,82]. A previous MR study also supported a causal effect of alcohol consumption in reducing TG [28]. The relationship between alcohol use and reduction in LDL-c is also concordant with two experimental studies involving red wine [14,15].

The IV analyses conducted in this study demonstrate a non-linear effect of alcohol consumption on TG, total cholesterol, HDL2-c, LDL-c, LDL-c, sdLDL-c, and apoB, with the highest effects observed at the third quartile of alcohol consumption. This suggests that alcohol consumption may have the greatest benefit within a low-to-moderate range. When excluding
### Table 4. Instrumental Variable analysis using 2SLS.

| Lipids                | N       | Predicted alcohol consumption quartiles† | β*   | 95% CI    | p a    | p overallb | 1st-stage partial R² | 1st-stage F-value |
|-----------------------|---------|-----------------------------------------|------|-----------|--------|------------|----------------------|------------------|
| TG                    | 9,911   | q1                                      | 0.00 |           | <0.001 | 0.12%      | 12.17                |                  |
|                       |         | q2                                      | -0.06|-0.09     | -0.02  | 0.001      |                      |                  |
|                       |         | q3                                      | -0.13|-0.20     | -0.07  | <0.001     |                      |                  |
|                       |         | q4                                      | -0.08|-0.17     | 0.00   | 0.049      |                      |                  |
| Total cholesterol     | 9,751   | q1                                      | 0.00 |           | <0.001 | 0.11%      | 10.86                |                  |
|                       |         | q2                                      | -5.54|-8.23    | -2.85  | <0.001     |                      |                  |
|                       |         | q3                                      | -7.71|-13.26   | -2.15  | 0.007      |                      |                  |
|                       |         | q4                                      | -4.56|-11.36   | 2.25   | 0.189      |                      |                  |
| HDL-c                 | 10,132  | q1                                      | 0.00 |           |         | 0.117      | 0.13%                | 13.48            |
|                       |         | q2                                      | 0.01 | -0.01    | 0.03   | 0.435      |                      |                  |
|                       |         | q3                                      | 0.04 | 0.00     | 0.07   | 0.070      |                      |                  |
|                       |         | q4                                      | 0.03 | -0.02    | 0.07   | 0.293      |                      |                  |
| HDL2-c                | 10,120  | q1                                      | 0.00 |           | <0.001 | 0.13%      | 13.48                |                  |
|                       |         | q2                                      | 0.04 | 0.00     | 0.07   | 0.040      |                      |                  |
|                       |         | q3                                      | 0.10 | 0.03     | 0.17   | 0.007      |                      |                  |
|                       |         | q4                                      | 0.06 | -0.03    | 0.15   | 0.179      |                      |                  |
| HDL3-c                | 10,120  | q1                                      | 0.00 |           |         | 0.916      | 0.13%                | 13.48            |
|                       |         | q2                                      | -0.19| -0.87  | 0.49   | 0.580      |                      |                  |
|                       |         | q3                                      | 0.08 | -1.23    | 1.38   | 0.908      |                      |                  |
|                       |         | q4                                      | 0.11 | -1.51   | 1.74   | 0.892      |                      |                  |
| LDL-c                 | 9,751   | q1                                      | 0.00 |           | <0.001 | 0.11%      | 10.86                |                  |
|                       |         | q2                                      | -4.60|-7.18   | -2.03  | <0.001     |                      |                  |
|                       |         | q3                                      | -6.87|-12.24  | -1.50  | 0.012      |                      |                  |
|                       |         | q4                                      | -4.57|-11.11  | 1.96   | 0.170      |                      |                  |
| sdLDL-c               | 8,102   | q1                                      | 0.00 |           |         | 0.054      | 0.07%                | 6.00             |
|                       |         | q2                                      | -0.04|-0.08   | -0.01  | 0.014      |                      |                  |
|                       |         | q3                                      | -0.08|-0.15   | -0.005 | 0.037      |                      |                  |
|                       |         | q4                                      | -0.08|-0.17   | 0.01   | 0.067      |                      |                  |
| apoB                  | 7,663   | q1                                      | 0.00 |           |         | 0.022      | 0.08%                | 6.13             |
|                       |         | q2                                      | -0.03|-0.04   | -0.01  | 0.005      |                      |                  |
|                       |         | q3                                      | -0.04|-0.07   | 0.001  | 0.058      |                      |                  |
|                       |         | q4                                      | -0.04|-0.08   | 0.01   | 0.132      |                      |                  |
| Lp(a)                 | 9,924   | q1                                      | 0.00 |           |         | 0.578      | 0.14%                | 13.87            |
|                       |         | q2                                      | -0.02|-0.09   | 0.06   | 0.657      |                      |                  |
|                       |         | q3                                      | -0.05|-0.20   | 0.10   | 0.500      |                      |                  |
|                       |         | q4                                      | -0.01|-0.20   | 0.18   | 0.890      |                      |                  |

† Quartile 1: 1.49–3.63 g/wk, quartile 2: 3.63–4.66 g/wk, quartile 3: 4.66–10.57 g/wk, and quartile 4: 10.57–19.54 g/wk,
* second stage regression coefficient between lipid measures and predicted alcohol consumption quartiles with quartile 1 as the reference group,
* Wald p-value comparing each quartile with the quartile 1,
* Wald p-value for overall effect of alcohol consumption,
* In transformed,
* measured at visit 4

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Table 5. Sensitivity IV analysis excluding heavy drinkers.

| Lipids                | N     | Predicted alcohol consumption quartiles | β*  | 95% CI | p   | p overallb | 1st-stage partial R² | 1st-stage F-value |
|-----------------------|-------|-----------------------------------------|-----|--------|-----|-----------|----------------------|------------------|
| TG                    | 9,024 | q1                                      | 0.00| <0.001 | 0.10%| 9.41      |                      |                  |
|                       |       | q2                                      | -0.06| -0.10  | -0.03| <0.001    |                      |                  |
|                       |       | q3                                      | -0.15| -0.22  | -0.08| <0.001    |                      |                  |
|                       |       | q4                                      | -0.11| -0.19  | -0.02| 0.018     |                      |                  |
| Total cholesterol     | 8,877 | q1                                      | 0.00| <0.001 | 0.10%| 8.64      |                      |                  |
|                       |       | q2                                      | -5.42| -8.21  | -2.63| <0.001    |                      |                  |
|                       |       | q3                                      | -6.43| -12.45 | -0.40| 0.037     |                      |                  |
|                       |       | q4                                      | -2.78| -10.07 | 4.51 | 0.454     |                      |                  |
| HDL-c                 | 9,228 | q1                                      | 0.00| 0.164  | 0.11%| 10.39     |                      |                  |
|                       |       | q2                                      | 0.01| -0.01  | 0.03 | 0.236     |                      |                  |
|                       |       | q3                                      | 0.04| 0.00   | 0.07 | 0.079     |                      |                  |
|                       |       | q4                                      | 0.03| -0.02  | 0.08 | 0.252     |                      |                  |
| HDL2-c                | 9,216 | q1                                      | 0.00| 0.002  | 0.11%| 10.38     |                      |                  |
|                       |       | q2                                      | 0.04| 0.00   | 0.07 | 0.034     |                      |                  |
|                       |       | q3                                      | 0.09| 0.01   | 0.16 | 0.023     |                      |                  |
|                       |       | q4                                      | 0.05| -0.04  | 0.14 | 0.277     |                      |                  |
| HDL3-c                | 9,216 | q1                                      | 0.00| 0.963  | 0.11%| 10.38     |                      |                  |
|                       |       | q2                                      | -0.01| -0.69  | 0.68 | 0.989     |                      |                  |
|                       |       | q3                                      | 0.23| -1.11  | 1.56 | 0.737     |                      |                  |
|                       |       | q4                                      | 0.37| -1.29  | 2.03 | 0.662     |                      |                  |
| LDL-c                 | 8,877 | q1                                      | 0.00| <0.001 | 0.10%| 8.64      |                      |                  |
|                       |       | q2                                      | -4.45| -7.10  | -1.80| 0.001     |                      |                  |
|                       |       | q3                                      | -4.95| -10.72 | 0.82 | 0.093     |                      |                  |
|                       |       | q4                                      | -2.29| -9.24  | 4.66 | 0.519     |                      |                  |
| sdLDL-c               | 7,517 | q1                                      | 0.00| 0.098  | 0.07%| 5.08      |                      |                  |
|                       |       | q2                                      | -0.04| -0.07  | 0.00 | 0.049     |                      |                  |
|                       |       | q3                                      | -0.08| -0.16  | -0.01| 0.031     |                      |                  |
|                       |       | q4                                      | -0.09| -0.18  | 0.00 | 0.060     |                      |                  |
| apoB                  | 7,110 | q1                                      | 0.00| 0.045  | 0.07%| 5.17      |                      |                  |
|                       |       | q2                                      | -0.02| -0.04  | 0.00 | 0.013     |                      |                  |
|                       |       | q3                                      | -0.04| -0.07  | 0.00 | 0.064     |                      |                  |
| Lp(a)                 | 9,040 | q1                                      | 0.00| 0.742  | 0.12%| 10.43     |                      |                  |
|                       |       | q2                                      | -0.02| -0.10  | 0.06 | 0.657     |                      |                  |
|                       |       | q3                                      | -0.05| -0.21  | 0.10 | 0.502     |                      |                  |
|                       |       | q4                                      | -0.03| -0.23  | 0.17 | 0.782     |                      |                  |

*second stage regression coefficient between lipid measures and predicted alcohol consumption quartiles with quartile 1 as the reference group,

aWald p-value comparing each quartile with the quartile 1,

bWald p-value for overall effect of alcohol consumption,

* In transformed,

# measured at visit 4

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heavy drinkers, the effect of alcohol consumption on those lipids remained significant, and with the peak effect at the third quartile. Therefore we conclude that alcohol consumption may have the greatest benefit within a low-to-moderate range and higher drinking does not always result in larger benefit. The consistent results when excluding never and former drinkers further confirm that the causal role of alcohol consumption on TG, total cholesterol, HDL2-c, LDL-c, sdLDL-c, and apoB are robust to potential confounding and reverse causation from adverse health conditions that may prevent people from drinking.

These findings may help explain the mechanism of a cardiovascular protective effect of alcohol consumption. Studies found HDL2-c, which are large HDL particles, had cardioprotective effect [83–85]. Alcohol consumption may raise HDL-c level by increasing hepatic production or increasing transport rate of apoA-I and apoA-II [7,86,87], increasing cellular cholesterol efflux and plasma cholesterol esterification [7,87,88], increasing muscle ATP-binding cassette, subfamily A (ABCA1) which may be important in recycling preformed HDL through reverse cholesterol transport, and decreasing cholesteryl ester transfer protein (CETP) [7,87]. Lowered CETP level was found associated with an increased level of large HDL particles [89]. sdLDL is considered a pro-atherogenic particle due to their susceptibility to oxidation that promotes inflammation and plaque development [44,90–92]. Studies suggest that apoB may be more predictive than LDL-c for the risk of CHD [91,93,94], and total apoB likely reflect the total number of atherogenic particles [92].

This study has a number of strengths. With the large sample size, this is one of the most comprehensive MR studies involving alcohol use and a comprehensive set of lipid measures. This is also the first MR study looking at the causal effect of alcohol consumption on HDL-c subfractions, sdLDL-c, apoB, and Lp(a). Compared to a case-control study, the cohort design helps to avoid selection bias and increases the validity of the MR approach [19]. This study employed a stringent process of selecting genetic instruments. The instrumental variants were examined by a thorough procedure of validating MR assumptions and possible violations including linkage disequilibrium (LD) and pleiotropy issues. The potential for population stratification was also addressed by controlling for genetic principal components in the IV regression models. The fact that the score was created from different genes further strengthens the causal inference in this study, because potential violations of MR assumptions through LD and pleiotropy issues were unlikely [23].

A limitation of this study is the fact that sdLDL-c and apoB were measured at visit 4 and the sample sizes were reduced compared to the analyses of other lipid measures at baseline. This resulted in F-values less than 10, indicating that the results have the potential for bias, and therefore the causal inference for sdLDL-c and apoB should be interpreted with caution. Several instrumental SNPs were related to alcohol dependence and alcoholism in previous studies. Given that the variants of interest are located in the ADH genes, the likely biological mechanism of the mutated alleles is to increase acetaldehyde level and prevent people from drinking. Therefore, those ADH genetic variants should influence alcohol consumption level, not just the status of dependence. This was confirmed by significant associations between the genetic score and alcohol consumption at baseline as well as at visit 4 (%2 Table).

In conclusion, this study supports the role of low-to-moderate alcohol use in improving lipid profiles. Continued investigation of the role of alcohol consumption on TG, total cholesterol, HDL2-c, LDL-c, sdLDL-c, and apoB is warranted.

Supporting Information

S1 Table. Genetic instrument selection (DOCX)
S2 Table. Association between unweighted genetic score and alcohol consumption
(DOCX)

S3 Table. Sensitivity IV analysis excluding never drinkers
(DOCX)

S4 Table. Sensitivity IV analysis excluding never and heavy drinkers
(DOCX)

S5 Table. Sensitivity IV analysis excluding never and former drinkers
(DOCX)

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Author Contributions
Conceived and designed the experiments: KNV EB ACM. Performed the experiments: RCH CMB KAV. Analyzed the data: KNV ACM. Contributed reagents/materials/analysis tools: RCH CMB EB. Wrote the paper: KNV CMB VN KAV EB ACM.

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