Using Mendelian randomization to evaluate the effects of alcohol consumption on the risk of coronary heart disease

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Abstract

Purpose – *This paper aims to evaluate the use of Mendelian randomization (MR) analyses for judging the effects of alcohol consumption on the risk of coronary heart disease (CHD).*

Design/methodology/approach – *This paper presents a review of methodology for MR and describes its early application to judging health effects of alcohol, current uses and a recommended approach of combining MR results with those from observational and experimental studies.*

Findings – *Early applications of MR to health effects of alcohol consumption were inadequate for providing unbiased results, but newer attempts using polygenic scores show promise. It is important to combine data from MR analyses with those from observational and experimental studies to obtain an unbiased and scientifically sound estimate of alcohol's effects on health.*

Practical implications – *Giving advice to the public regarding alcohol consumption must be based on accurate, unbiased scientific data; this paper describes attempts to use MR for achieving this goal.*

Social implications – *Given that light-to-moderate alcohol intake is associated with a lower risk of CHD, type II diabetes mellitus and total mortality, it is important to be able to evaluate both the benefits and harms from alcohol before giving advice regarding drinking.*

Originality/value – *This is part of a group of three papers dealing with the potential health benefits and harms associated with alcohol consumption.*

Keywords *Alcohol, Mortality, Coronary, J-shaped curve, Mendelian*

Paper type *General review*

Introduction

Approaches for estimating health effects of alcohol consumption

Almost all data associating alcohol intake with risk of coronary heart disease (CHD) come from observational studies, with self-report of consumption. The observational evidence is strongly supported by short-term trials of alcoholic beverage consumption for their relation to cardiovascular risk factors, and by a vast amount of experimental data in animals. However, what has not been carried out is a large and long-term trial administering alcohol to healthy individuals and then waiting for the initial development of CHD; in our opinion, such a randomized clinical trial might not be feasible.

Some scientists have suggested using Mendelian randomization (MR) as an alternative way of obtaining unbiased "truth" about the association between alcohol and CHD, using genetic factors as indices of alcohol intake. There are many difficulties in interpreting such studies, as alcohol use and abuse are known to be extremely complex in that they are related not only to genetic influences but also to many lifestyle and other environmental factors.

In this paper, we will first describe briefly what MR consists of. We will then summarize first what observational cohort studies have shown about the relation of alcohol to certain types

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of cancer and to CHD before giving some results of early MR studies related to cancer (of which there are considerable data) and to CHD. The studies related to CHD were initially carried out using only polymorphisms that relate to a single biologic effect (e.g. the metabolism of alcohol from specific enzymes such as alcohol dehydrogenase), but more recent analyses have used a polygenic score incorporating many genetic factors for their relation to CHD. Finally, we will review some current ideas about the future use of MR, in combination with data from observational and experimental studies, for obtaining more accurate and unbiased estimates of the relation of alcohol to CHD.

Results/discussion

What are the main components of Mendelian randomization?

MR takes a genetic factor, or group of factors, that relate to alcohol consumption and uses it/them to serve as an instrumental variable (IV), a proxy for exposure to alcohol, in an attempt to judge the effects of alcohol in a way that is less likely to be biased or confounded than having to rely only on self-reported consumption data. The IV for MR analyses must be correlated with the exposure (alcohol consumption) but cannot be associated with any confounder of the exposure–outcome relation, nor with any pathway by which the IV can influence the outcome other than via the exposure of interest. Genetic variants used as IVs in MR studies of the effects of alcohol consumption were initially single-nucleotide polymorphisms (SNPs) related to the metabolism of alcohol or its breakdown product, acetaldehyde. The main difficulty in any MR analysis is the choice of an appropriate IV and the assessment of the IV assumptions; if the IV assumptions do not hold, then inferences from any analysis will be unreliable (Burgess *et al.*, 2017).

To be more specific, for valid causal conclusions, each genetic variant used in a MR analysis must be associated only with the risk factor of interest, level of alcohol intake in the present case, and be independent of all confounding variables that may affect the risk of CHD by means other than through alcohol intake. MR is supposed to be less likely to be affected by bias or confounding than conventional observational studies, which rely on individuals giving an accurate report of their current and former drinking habits (which we will show must include not only the number of drinks/week but especially the pattern of drinking). MR analyses depend on an accurate and unbiased measure of alcohol exposure as the IV, which depends on a number of plausible assumptions; these assumptions must be supported by data from a variety of sources before being used for making clinical decisions (Davies *et al.*, 2018).

If using an ideal IV, MR would be the equivalent of a randomized controlled trial (RCT). While a large RCT evaluating the effects of alcohol intake on cardiovascular disease or death has not been done, a number of RCTs have been done for assessing the effects of alcohol consumption on cardiovascular risk factors. For example, over many decades essentially all studies of alcohol administration have shown a direct effect on the level of HDL-cholesterol (HDL-C) concentrations [e.g., Burr *et al.* (1986); de Oliveira e Silva, *et al.* (2000)]. HDL-C levels may decrease over time, but in a prospective study, Huang *et al.* (2017) reported that over a 6-year period, moderate drinkers had the least decrease in HDL-C over time when compared with non-drinkers and heavy drinkers.

Alcohol consumption may also affect the risk of CHD through beneficial effects on blood clot formation and fibrinolysis, as well as on platelet aggregation. For example, Rubin (1999) summarized experimental data showing that alcohol decreased platelet aggregability. Sierksma *et al.* (2002) demonstrated that moderate alcohol consumption significantly decreased plasma C-reactive protein and fibrinogen levels; this led the authors to conclude: "An anti-inflammatory action of alcohol may help explain the link between moderate alcohol consumption and lower cardiovascular disease risk." In another RCT, Gepner *et al.* (2015) demonstrated improvement in diabetes control among subjects

randomly assigned to consume alcohol. Chiva-Blanch *et al.* (2015) demonstrated that the phenolic content of beer reduces leukocyte adhesion molecules and inflammatory biomarkers, whereas alcohol mainly improves the lipid profile and reduces some plasma inflammatory biomarkers related to atherosclerosis.

How good are self-reports of alcohol intake in observational studies?

Before MR analyses are compared with self-reported alcohol consumption, it is important to judge the degree to which the latter provides a valid estimate of intake. It must be stated at the onset that the use of self-reported data on alcohol intake has been amazingly consistent in showing a J-shaped relation with cardiovascular disease across diverse populations over many decades, as demonstrated in key meta-analyses by Di Castelnuovo *et al.* (2006) and Ronksley *et al.* (2011).

There are always inconsistencies among observational studies of alcohol and disease entities which may be due to several methodological issues: underreporting of alcohol by drinkers may be common, and this could result in an increased risk of adverse outcomes from reportedly small amounts of alcohol. Another key issue is the alcohol drinking *pattern*. The average amount of alcohol consumed must always be considered, but the pattern of alcohol consumption may be even more important for the relation with cancer; further, binge drinking has also been shown to abolish the protective effect against CHD seen with moderate drinking (Mukamal *et al.*, 2020a). In general, any investigation that focuses only on average alcohol intake should not be considered as providing adequate data on the net effect of alcohol on any health outcome, and this includes observational studies and those using MR.

Studies of alcohol's relation to cancer and coronary heart disease

There may be important differences between relating alcohol consumption to CHD than when relating alcohol intake to cancer. The extensive data available on alcohol and cancer provide insights into the use of MR that are relevant for evaluating the association with CHD as well. For example, binge drinking in women is associated with an increased incidence of breast cancer (White *et al.*, 2017), while the measurement of only "alcohol intake per week" (used in most observational and MR studies) often does not account for binge drinking. Mørch *et al.* (2007) emphasized the need to evaluate the *pattern* of drinking, rather than just the average intake, as they found that weekend drinking among women had a stronger effect on breast cancer risk than similar amounts when consumed throughout the week. These authors stated, "For alcohol consumption above the intake most frequently reported (1–3 drinks/day), the risk of breast cancer is increased; a possible threshold in risk estimates was found for consumption above 27 drinks per week. The risk is minor for moderate levels but increases for each additional drink consumed during the week. Weekend consumption and binge drinking imply an additional increase in breast cancer risk."

For cancer studies, especially, peak blood levels of alcohol (as seen with binge drinking of large amounts of alcohol in a short time) rather than average weekly amount may be the more important measure, as higher blood alcohol levels may be key in raising levels of the main alcohol break-down product, acetaldehyde, a known mutagen (Seitz and Stickel, 2010); alcohol *per se* is neither genotoxic nor mutagenic (Salaspuro, 2020). Further, measures of excessive alcohol consumption (such as indices of alcoholism or other alcohol use disorders), provide additional information on risk. We postulate that the relation of alcohol intake to cancer may tend to be linear (perhaps related to peak blood levels of alcohol, as from heavy binge drinking), while the evidence is strong that the relation to cardiovascular diseases is non-linear,

usually a J-shaped curve: a lower risk for light-to-moderate intake but increased risk with consistent heavy drinking or binge drinking (Forum Critique 241).

Key observational studies relating alcohol and coronary heart disease incidence and mortality

Before judging the value of MR studies in estimating risk of CHD associated with alcohol, we must briefly describe the role of observational studies. In an associated paper in this issue of the journal, Skovenborg *et al.* (2021) describe the work of Raymond Pearl in 1926, presenting a very early observation of decreased mortality among consumers of alcohol in comparison with abstainers. An excellent, more modern, review of the topic of alcohol and disease was published by Moore and Pearson (1986) in 1986, who found an inverse dose-response relationship between alcohol intake and CHD which was U-shaped or J-shaped in most studies. In 2006, a meta-analysis of 34 prospective studies, pooling findings from more than one million individuals and almost 100,000 deaths, showed a J-shaped relationship between alcohol intake and total mortality. Consumption of up to 2 drinks per day in women and up to 4 drinks per day in men was associated with lower mortality than zero consumption, with about one-half drink per day associated with the lowest mortality risk (Di Castelnuovo *et al.*, 2006).

A widely quoted more recent paper on the topic, by Ronksley *et al.* in 2011, was based on a meta-analysis of 84 studies; in their analyses, the pooled adjusted relative risks for alcohol drinkers relative to non-drinkers in random effects models for CHD incidence was OR= 0.71 (0.66–0.77). It is considered that reductions in risk of CHD and total mortality associated with light-to-moderate alcohol intake are among the most consistent findings of any biological relationship studied.

Use of Mendelian randomization for evaluating alcohol and coronary heart disease

Stating concern about bias and uncontrolled confounding from self-reports of alcohol intake in observational studies, some investigators have considered that MR may be a better approach for studying the effects of alcohol consumption on the risk of CHD. Using SNPs related to alcohol dehydrogenase (ADH), or other factors related to alcohol metabolism, would be expected to be a potential IV for such studies. The allele ADH1B*1 encodes for a slow-oxidizing enzyme in the ADH1B gene, while ADH1B*2, encodes for a fast-oxidizing enzyme that has about 100 times the oxidation capability of the ADH1B*1 allele (Bosron *et al.*, 1983). The ADH1B*2 allele is associated with a rapid increase in blood levels of acetaldehyde, the toxic break-down product of alcohol, which causes a flushing response to intake of alcohol. People with this allele usually report a reduced maximum number of drinks in one sitting and a lower risk of alcohol dependence (Hurley and Edenberg, 2012). The ADH1B*2 allele is found in 80% or more of northeast Asians (i.e. Chinese, Japanese, and Koreans) but in only 10% or less of Caucasians of European ancestry, mainly those of Semitic ancestry (Goedde *et al.*, 1992; Osier *et al.*, 2002). The latter, Jews and Arabs, typically also have very different behaviors regarding alcohol consumption, environmental factors that could lead to confounding.

A widely quoted attempt at using variations in the ADH gene in a MR analysis for judging the effects of alcohol on CHD risk, reported by Holmes *et al.* in 2014, was based on a meta-analysis of 56 epidemiological studies (261,991 individuals of European descent, including 20,259 CHD cases and 10,164 stroke events). Carriers of the (fast-metabolizing) ADH1B*2 allele reported 17.2% fewer units of alcohol per week, had 22% lower prevalence of binge drinking, and had higher abstention rates (OR 1.27) than non-carriers. These authors reported lower odds of CHD (OR 0.90 [95% CI 0.84–0.96]) among these ADH1B*2 allele carriers. However, the authors went on to conclude that "Individuals with a genetic variant associated with non-drinking and lower alcohol consumption had a more favorable

cardiovascular profile and a reduced risk of CHD than those without the genetic variant", leading them to the conclusion that there was no protective effect of moderate drinking on the risk of coronary disease. Unfortunately, this conclusion was based on exactly the same type of information bias that the MR study method should avoid.

Many scientists raised serious questions about the conclusions of the authors of the paper by Holmes *et al.* For example, 17 rapid responses were published by BMJ, most of which noted deficiencies in the paper and expressed strong disagreement with the conclusions of the authors (BMJ, Rapid Responses, 2014; https://www.bmj.com/content/349/bmj.g4164/rapid-responses; published 26 May 2015, accessed 13 August 2020). It was pointed out by many that the sole use of the ADH1B allele violates a key assumption required for an IV: that it must have no effects except its relation to the exposure of interest (alcohol intake) (Forum Critique 143). Further, the Holmes *et al.* paper found little relation of their estimate of alcohol consumption with HDL-cholesterol, while essentially all observational studies, clinical trials, and experimental studies over many decades have shown that alcohol is an important determinant of HDL (Ernst *et al.*, 1980). Also, Holmes *et al.* do not consider the pattern of drinking. Thus, the strong consensus of reviewers of that study considered that it does not provide reliable data upon which to judge the overall effects of alcohol on the risk of CHD.

An attempt to use a combination of the genes ADH1B and aldehyde dehydrogenase as the instrumental variable in a MR

Alcohol is oxidized to acetaldehyde, which in turn is oxidized by aldehyde dehydrogenases (ALDHs) to acetate. Compared to the typical form of ALDH2 (ALDH2*1/1) the ALDH2-rs671 polymorphism (ALDH2*2) has decreased enzyme activity. ALDH2 encoded by heterozygotes of the genetic polymorphism (ALDH2*1/2) maintains only 30–40% of full enzymatic activity, while ALDH2 encoded by polymorphism homozygotes (ALDH2*2/2) shows negligible activity causing a flush response and other adverse responses to alcohol consumption (Wall *et al.*, 2016). The prevalence of (ALDH2*2) is about 30%–50% in the Asian population, while it is rare in Caucasian populations. ALDH2 is the primary and most efficient enzyme to detoxify toxic aldehydes *in vivo*, and a meta-analysis of 12 case-control studies by Han *et al.* (2013) strongly suggested that the ALDH2-rs671 polymorphism was associated with increased risks of both CHD and myocardial infarction. These effects may affect the outcome through another trait or pathway to the one under investigation (the effects of alcohol on CHD risk) which is a clear violation of the instrumental variable assumptions (Davies *et al.*, 2018).

In the prospective China Kadoorie Biobank study (Millwood *et al.*, 2019), 161,498 participants were genotyped for two variants that alter alcohol metabolism, ALDH2-rs671 and ADH1B-rs1229984. Men who reported drinking about 100 g of alcohol per week (one to two drinks per day) had lower risks of ischaemic stroke, intracerebral haemorrhage, and acute myocardial infarction than non-drinkers or heavier drinkers. While genotype-predicted mean alcohol intake among males varied widely (from 4 to 256 g per week), it did not have a J-shaped or U-shaped association with risk of myocardial infarction, considered by the authors to weaken the J-shaped relation shown in most observational studies.

Many scientists have questioned the conclusions of these authors, stating that the majority of the Chinese cohort drank only spirits, which may have different effects on CHD than beer or wine, which are more commonly consumed in the west (Costanzo *et al.*, 2011). Further, drinking pattern was not considered, and previous research suggests that male drinkers in China average rather large amounts of alcohol, which they often consume in binges (Li *et al.*, 2011). Further, the two alleles studied have been shown to affect more than just alcohol consumption, limiting their use as an IV (Chen *et al.*, 2014). Therefore, again, inferences from this study are limited.

The conclusion that one or two genes are inadequate to judge alcohol intake in MR analysis: Unlike situations in which a single dominant genetic factor leads invariably to a

disease (e.g. Huntington's disease), most scientists now realize that single or very few gene variants are inadequate to use as an IV in evaluating alcohol intake, which is a very complex behavioral lifestyle factor. This was stated by Wall *et al.* (2016) when they evaluated a number of genes affecting alcohol metabolism: "However, these alleles do not act in isolation to influence the risk of alcohol use disorders. Moreover, other factors have been found to influence the extent to which these alleles affect a person's alcohol involvement, including developmental stage, individual characteristics (e.g. ethnicity, antisocial behavior, and behavioral undercontrol), and environmental factors (e.g. culture, religion, family environment, and childhood adversity)." More recent MR studies on this topic have used the effects of multiple genes combined into a polygenic score as the IV to study the relation of alcohol to CHD.

Even the use of combining a small number of genes affecting alcohol metabolism as an IV has problems. For example, the presence of the ADH1C*2 allele would be associated with a slightly reduced oxidizing capacity, whereas the presence of ADH1B*2 and ADH1B*3 alleles would be associated with a substantially higher oxidative capacity (i.e. more rapid ethanol oxidation to acetaldehyde). These calculations are rough approximations, however, because they assume that the different alleles are expressed at equal levels. In reality, other factors, including liver size and differences in gene expression, can lead to differences even between individuals carrying the same alleles (i.e., with the same genotype). For example, among healthy young European Americans, almost all of whom are homozygous for ADH1B*1, actual ethanol elimination rates have been shown to differ about four-fold (Edenberg, 2007). It must be concluded that both average alcohol consumption and drinking patterns interact in complex ways with regard to CHD risk, and the alcohol-heart relationship cannot be accurately described using only one of the dimensions of alcohol intake.

Combining effects of multiple genes into a polygenic score to use as an instrumental variable

Scientists have begun to consider the combination of a large number of genetic factors that relate to an outcome of interest (e.g. CHD) and have generated polygenic scores. Overall, it is clear that increasing the number of factors included in a polygenic score can provide an improved IV variable, as it may be used to find SNPs associated with specific patterns of alcohol consumption, a major limitation of IV thus far.

Examples of the use of polygenic scores from recent publications include that of Larsson *et al.* (2020). These investigators used up to 94 SNPs relating to alcohol consumption, with their selection of variables based especially on genetic factors affecting alcohol use; however, their SNPs also related to some risky behaviors, cannabis use, and especially to smoking (an association described below by Liu *et al.*, 2019). Given that valid instrumental variables are defined by assumptions:

- that they associate with the risk factor of interest (the relevance assumption);
- that they share no common cause with the outcome (the independence assumption); and
- that they do not affect the outcome except through the risk factor (the exclusion restriction assumption), the genetic association found with risky behavior and cannabis use by Larson *et al.* may be a violation of the exclusion restriction assumption.

An important paper by Liu *et al.* (2019) studied genetic variables for both tobacco and alcohol use among 1.2 million individuals; they discovered 566 genetic variants in 406 loci associated with multiple stages of tobacco use (initiation, cessation, and heaviness) as well as for alcohol use, with 150 loci evidencing pleiotropic association. The authors found loci that affected multiple substance use phenotypes (such as measures of smoking, alcohol consumption, years of education, depressive disorder, BMI, cholesterol, CHD, kidney disease, and other

conditions, and included genes involved in nicotinic, dopaminergic and glutamatergic neurotransmission). Their results provide a solid starting point to evaluate the effects of these loci in model organisms and more precise substance use measures. Liu *et al.* concluded: "Ultimately, substance use is embedded in a complex web of causal relations and caution must be exercised in drawing strong causal conclusions from a study of a single risk factor. However our findings represent a major step forward in understanding the etiology of these complex, disease-relevant, behaviors." We agree with the authors that such approaches could be very important in teasing apart genetic factors associated with alcohol consumption and, as stated by the authors, "[…] will aid in the discovery of mechanisms by which implicated genes may affect substance use and related disease risk."

Observational studies relating alcohol intake to risk of breast cancer

Observational and MR studies of the relation of alcohol consumption to a variety of cancers provide information that applies also to the risk of CHD. The effect of alcohol intake on breast cancer has created much public interest as in most cohort studies there has been found to be an increase in risk even for light drinking. In a meta-analysis of observational epidemiologic studies in 1998, Smith-Warner *et al.* demonstrated a positive relation of alcohol consumption to the risk of breast cancer, even among women reporting no more than up to 1 drink/day. Similarly, Seitz *et al.* (2012) meta-analyzed 113 studies with 122,091 cases and identified a modest but significant association between light drinking ($\leq$12.5 g/day ethanol; $\leq$1 drink/day) and breast cancer (RR = 1.04, 95% CI: 1.02–1.07). These authors concluded: "A significant increase of the order of 4% in the risk of breast cancer is already present at intakes of up to one alcoholic drink/day. Heavy alcohol consumption, defined as three or more drinks/day, is associated with an increased risk by 40–50%."

Dam *et al.* (2016) evaluated the association between *changes* in alcohol consumption and breast cancer risk in a large prospective cohort of 21,523 postmenopausal women and found that women reporting increases between 1 and 2 drinks per day had a 13% higher risk of breast cancer when compared with women reporting an increase of no more than 1 drink/day. They stated, "Analyses modeling five year change in alcohol intake with cubic splines showed that women who increased their alcohol intake over the five year period had a higher risk of breast cancer and a lower risk of CHD than women with a stable alcohol intake."

The Nurses' Health Study was one of the first large epidemiological cohort studies to demonstrate an increase in risk of breast cancer to be associated with alcohol consumption [and contributed data to the Smith-Warner *et al.* (1998) paper]. An updated summary report from that study was published by Chen *et al.* (2011). Those authors conclude: "In summary, our study provides a comprehensive assessment of the relationship between alcohol intake and breast cancer risk in terms of timing, frequency, quantity, and types of alcohol in a large prospective cohort with detailed information on breast cancer risk factors. We did find an increased risk at low levels of use, but the risk was quite small. However, an individual will need to weigh the modest risks of light to moderate alcohol use on breast cancer development against the beneficial effects on cardiovascular disease to make the best personal choice regarding alcohol consumption." Other reports from the Nurses' Health Study (Mostofsky *et al.*, 2016) support the findings of a slight increase in breast cancer risk, but a lower risk of total mortality, for light-moderate drinkers when compared with non-drinkers or heavier drinkers. An overview on the topic of alcohol and breast cancer has recently been published by Freudenheim (2020).

Using Mendelian randomization to judge the risk of certain types of cancer associated with alcohol consumption

While this paper focuses on CHD, the use of MR for evaluating cancer may provide relevant information. For example, in an early MR study relating alcohol to the risk of esophageal

cancer, Lewis and Smith (2005) aggregated summary results from seven studies with a total of 905 cases of esophageal cancer and provided strong evidence that the risk of esophageal cancer was lower among ALDH2*2 homozygotes (OR = 0.36; 95% CI = 0.16–0.80) as compared to ALDH2*1 homozygotes. This study gives a clear indication that certain ALDH2 alleles decrease the risk of esophageal cancer through their effects on alcohol consumption.

Kranzler *et al.* (2019) used MR to evaluate genetic factors associated with two measures of alcohol use: the three-item AUDIT-C, a continuous measure of the frequency and quantity of usual drinking and the frequency of binge drinking, and the 7-item AUDIT-P scale, which focuses on alcohol-related disorders. In a meta-analysis across five populations, these authors found 13 independent loci associated with AUDIT-C, five of which were previously associated with a self-reported measure of alcohol consumption. Similarly, for AUDIT-P, their trans-population meta-analysis identified 10 independent GWS loci for AUD.

Differences when using Mendelian randomization analyses for effects of light drinking versus heavy alcohol use/abuse

Taking the above studies into consideration, we believe that the study by Lewis and Smith (2005) may have shown a large effect of genetic factors on risk because esophageal cancer is related especially to heavy alcohol intake, while the endocrine cancers studied with MR by Zhu *et al.* (2020) were for breast and ovarian cancer, where the question was if these cancers were related to light-to-moderate consumption, and they found little association. Also, the relation between genetic factors and estimates of alcohol use/abuse from the AUDIT-P by Kranzler *et al.* might support a proposition that such indices may serve as stronger IVs for studies related especially to heavier drinking than to those related to light drinking.

This supposition is supported by a recent paper by Williams (2020) on the heritability of alcohol intake across two generations in the Framingham Heart Study, in which he reports that heritability of alcohol intake is greater for heavy drinkers than for light drinkers. Williams found that genetic heritability estimated from the offspring-parent regression slope increased significantly from lowest to highest reported grams/day of alcohol consumption (0.006 ± 0.001 per percent, $P = 1.1 \times 10 - 7$). Heritability at the 90th percentile of the sample distribution was 4.5-fold greater than at the 10th percentile. Thus, one would expect stronger genetic factors for outcomes associated especially with heavy consumption or abuse than for light drinking.

How can we utilize Mendelian randomization studies when evaluating the effects of alcohol on health?

Mukamal *et al.* (2020a) have been disturbed that early MR studies, including those based on a single genetic factor, have been touted at giving the "unbiased truth" about alcohol and health outcomes, making all observational and experimental studies unnecessary. They argue: "In recent years, epidemiologists have increasingly sought to employ genetic data to identify 'causal' relationships between exposures of interest and various endpoints – an instrumental variable approach sometimes termed MR. However, this approach is subject to all of the limitations of instrumental variable analysis and to several limitations specific to its genetic underpinnings, including confounding, weak instrument bias, pleiotropy, adaptation and failure of replication." On the other hand, a MR study of alcohol in the setting of a perfect IV would be expected to demonstrate the same effect as a RCT.

Mukamal *et al.* (2020a) continued, "Although the approach enjoys some utility in testing the etiological role of discrete biochemical pathways, like folate metabolism, examples like that of alcohol consumption and cardiovascular disease demonstrate that it must be treated with all of the circumspection that should accompany all forms of observational epidemiology." In another publication, Mukamal *et al.* (2020b) describe further restrictions on the use of using MR studies as the *only* approach for evaluating alcohol and disease. In contrast, Smith *et al.* (2020) have

continued to support the value of MR studies, but conclude their argument: "Going forward, rather than focus on nomenclature, let us move to a situation in which triangulation of findings becomes the norm in epidemiology, and methods are considered on the basis of what they have to add to a reliable evaluation of each particular question."

While not evaluating alcohol as a risk factor for CHD, two recent reports in JAMA comparing conventional risk factors, based on observational data, and with polygenic risk assessment from MR in huge datasets, provided interesting results. Mosley *et al.* (2020) found that the polygenic score did estimate the occurrence of CHD, but did not add significantly to the estimates using conventional risk factors. Elliott *et al.* (2020) found only a slight improvement in risk assessment when MR results were added to results using only conventional risk factors. In an accompanying editorial based on these studies in JAMA, Khan *et al.* (2020) concluded that "The available data do not support the clinical utility of CAD polygenic risk scores (in their current form) in middle-aged adults of European descent."

The future of Mendelian randomization for evaluating effects of alcohol on coronary heart disease

Undoubtedly, we will continue to find new genetic factors related to CHD and will be able to combine up to millions of additional SNPs into new polygenic scores (Musunuru and Kathiresan, 2019). These efforts will be enhanced by use of large datasets and large-scale biobank cohorts (Tada *et al.*, 2016). As more genetic factors are discovered and used for defining instrumental variables, we will be better able to combine data from observational studies, human and animal experiments, and MR studies as a way of including genetic factors in our efforts to learn the true relation between alcohol consumption and health. However, we appreciate that relying just on an estimate of average intake, however accurate, is insufficient to study this relation – many environmental and lifestyle factors are important in modifying the health effects of drinking, and must be considered when making conclusions or recommendations on alcohol and health.

Conclusions

There is little question that our knowledge of genetic factors that relate to CHD will progress markedly in the future. However, even though we have already identified many such factors that we can include in MR analyses, it is clear that results from a variety of types of studies must be considered when attempting to judge the overall health effects of alcohol consumption. This is especially the case because type of beverage, drinking patterns (e.g. regular moderate versus binge drinking, rate of consumption, with or without food), smoking and other lifestyle habits, diet and many other environmental factors can modify the effects of alcohol consumption. Thus, the combination of data from observational studies, clinical trials, animal experiments, as well as MR analyses, will be needed to improve our knowledge on the relation of alcohol intake to health and disease; it remains a continuing challenge.

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