

Mendelian randomization-inspired causal inference in the absence of genetic data

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Studying the long-term causal effects of alcohol drinking is notoriously difficult. Epidemiological studies that use conventional analytical approaches are likely to be confounded and affected by reporting/recall bias and reverse causality, specifically in the form of the sick quitter effect (individuals quitting or never starting to consume alcohol due to underlying ill-health)(1). Decades of observational data showing J-shaped relationships of alcohol with risk of disease and in particular cardiovascular disease(2), fuelled by confirmation bias, have resulted in alcohol policies such that individuals are recommended to drink in moderation, due to putative cardioprotective effects. Critically, RCTs to investigate the long-term effects of alcohol drinking are not feasible for reasons including lack of suitable and ethical interventions and extended duration (and hence cost and likely high loss to follow-up).

Complimentary approaches for causal inference that could mimic the effects of randomized allocation to alcohol exposure acting over prolonged periods of time can be used to provide guidance on the reliability of existing data. Mendelian randomization (MR) is one such approach: an instrumental variable analysis exploiting genotypes as instruments (Figure 1).(3)

Insert Figure 1 here

Specifically, variants exist in alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) genes that are associated with faster or slower metabolism and different patterns of accumulation of alcohol metabolites, and which in turn can confer protection from excessive alcohol consumption (e.g. a variant in *ADH1B*) or even result in virtual intolerance to alcohol in the most extreme of cases (e.g. homozygosity for the *2 variant in *ALDH2*, i.e. *ALDH2**2/*2 individuals) (Figure 2).(4) In theory, such MR analyses can provide causal estimates of life-long exposure lasting for at least as long as someone's history of alcohol use, by comparing individuals according to their relatively alcohol-prone or alcohol-averse genotype.

Insert Figure 2 here

Large-scale studies have used *ADH1B* genotype in Mendelian randomization analyses of European cohorts to make causal inference and have shown that increased alcohol consumption results in deleterious effects on several cardiovascular risk factors (including blood pressure, inflammation and adiposity) and an increased risk of coronary heart disease (CHD) events(5, 6). These CHD findings are in stark contrast to several observational studies and given that adequately-conducted MR studies provide greater validity for causal inference than conventional observational studies (Figure 1), recent European guidelines have incorporated these findings to downplay the likely beneficial effect of alcohol on CHD.(7)

One limitation of using *ADH1B* genotype to assess the causal effect of alcohol across the full spectrum of intake is that the *2 allele results in a reduction in consumption of only around 17%(5). In this regard, the *ALDH2* genotype, which has a profound effect on alcohol consumption, may give rise to more powerful causal inference. Thus far, *ALDH2* has been associated with a number of CVD traits including blood pressure(8) and lipids(9). However, this variant is only present in East Asian populations and only few East Asian cohort studies to date have the required genetic information to conduct MR analyses (due to lack of stored blood samples, patient consent and/or funds to cover genotyping), thus limiting the range of outcome phenotypes that can be investigated.

An alternative to conducting a classic MR analysis exists in the case of *ALDH2*. The genetic variant associated with *ALDH2* inactivity or reduced activity determines a clear phenotypic reaction: the

flush, which is almost a Mendelian trait, deriving from the inefficient clearing of acetaldehyde. In the absence of genotype data, and assuming a strong and specific association between flushing and the *ALDH2* variant, it may be possible to use alcohol flushing reaction as a proxy for genotype.

Insert Figure 3 here

Flushing was first used as a phenotypic proxy for the *ALDH2* genotype by Yokoyama et al in their study of the effects of alcohol on oesophageal cancer. (10) In the current issue, Yun et al used (11) flushing to assess the causal relationship of alcohol on cardiovascular risk factors and coronary artery calcification. Here and for the first time, a full instrumental variable analysis was carried out to estimate effect sizes associated with alcohol consumption. Their results, that alcohol consumption has a causal detrimental effect on several cardiovascular and metabolic traits including coronary artery calcification, are tantalising.

The validity of these important findings relies on the suitability of alcohol flushing as a proxy for *ALDH2* genotype and itself an instrumental variable for alcohol consumption levels. The authors report that the prevalence of flushing in their study is very similar to the prevalence of the alcohol-flushing *ALDH2* variant in similar studies of Korean populations. Furthermore, the reported distribution of alcohol flushing status by age, sex and potential confounders provide additional reassurance that the core instrumental variable assumptions are met with lack of association of alcohol flushing with demographic traits and absence of survival bias. Presenting such data is strongly recommended as optimal reporting of IV and MR analyses (12, 13), as is also routinely done in the reporting of RCTs (the conventional 'Table 1' to demonstrate that confounders are equally balanced across intervention and comparator arms), as a basic check of the assumption that an instrumental variable is not associated with the potential confounders of the exposure-outcome association.

One of the complications to bear in mind when interpreting results from this and other studies using alcohol flushing as an instrumental variable-proxy for genotype, is related with the implicit assumption that *ALDH2* variation behaves in an additive or dominant way with respect to the outcome of interest (i.e. that heterozygote individuals (*1/*2) display either intermediate levels of risk compared to the two homozygote groups -additive- or the same level of risk as the rare *2/*2

homozygotes -dominant). This is important as evidence indicates possible violations of this assumption, e.g. the association of *ALDH2* genotype with oesophageal cancer departs from additivity and instead shows a “qualitative” interaction with alcohol drinking.(14) When using flushing as a proxy for *ALDH2* genotype, individuals who are heterozygotes (*1/*2) and homozygotes (*2/*2) for the loss of function allele *ALDH2**2 are grouped together in the same instrumental variable category as alcohol flushing behaves as a dominant trait with respect to *ALDH2* variation (Table 1). However, heterozygotes experience only some of the undesirable effects of drinking alcohol due to some degree of acetaldehyde build-up, but often they are still able to drink and some do consume considerable amounts of alcohol. For alcohol, the overall consumption will be lower in flushers than in the non-flushers (as *1/*2 carriers drink less than *1/*1 carriers and *2/*2 carriers drink almost no alcohol). However for acetaldehyde, the picture is more complex.(15) Flushers will consist of two very different groups with respect to acetaldehyde concentrations: heterozygotes (*1/*2) that consume alcohol and have markedly high concentrations and *2 homozygotes who generally don’t drink and have minimal concentrations of acetaldehyde as a result. Non-flushers (proxying *1/*1 genotype) have normally functioning *ALDH2*, drink normally and can clear acetaldehyde (Table 1).

Insert Table 1 here

Therefore, flushing is a valid instrument if ethanol itself is the supposed causal risk factor, since flushers will tend to have reduced risk of (alcohol-related) disease than non-flushers because of their reduced alcohol consumption, even though the effect will be diluted compared to the risk observed for *ALDH2* *2/*2 vs *ALDH2* *1/*1. On the other hand, if acetaldehyde is the hypothesised causal risk factor, then flushers could have lower, similar or even higher risk of disease, depending on genotype frequencies and how much heterozygotes drink in relation to *ALDH2**1*1. The latter situation typically happens for certain upper aerodigestive tract cancers such as oesophageal cancer(14) and in this scenario, flushing would be unlikely to be a valid proxy for genotype, as it does not allow a more refined analysis by *ALDH2**2 status. In the study by Yun et al,(11) we would expect the effect to follow an additive relationship given that alcohol would be the likely causal agent for coronary artery calcification (vs acetaldehyde), and therefore the flushing phenotype should be an acceptable proxy for *ALDH2* genotype and in turn for mean ethanol exposure (Table 2).

Insert Table 2 here

In summary, Yun et al's findings of a detrimental effect of alcohol on coronary artery calcification(11) provide further evidence against a cardioprotective role of alcohol. The authors' novel approach also highlights the potential to achieve pragmatic but rigorous Mendelian randomization-inspired causal inference in the absence of genetic data, by using a phenotypic proxy of a genotype in instrumental variable analyses. Future studies could use similar approaches, perhaps with genotype data in a subpopulation to better validate the phenotypic proxy. Of note, this example is rather unique – the trait being essentially Mendelian and thus guaranteeing high degrees of specificity for the genotype (confounding of the instrumental variable-outcome association is minimised), as well as good strength of association (low risk of 'weak instrument bias'). .

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Figure 1. Hierarchy of evidence. Mendelian randomization sits at the interface between observational and interventional epidemiology. In cases where RCTs are not feasible or practical and also, importantly, prior to embarking on new RCTs (and thus to mitigate risk of a negative outcome), MR analyses can provide reliable evidence on the causal relationship between exposures and risks of disease.

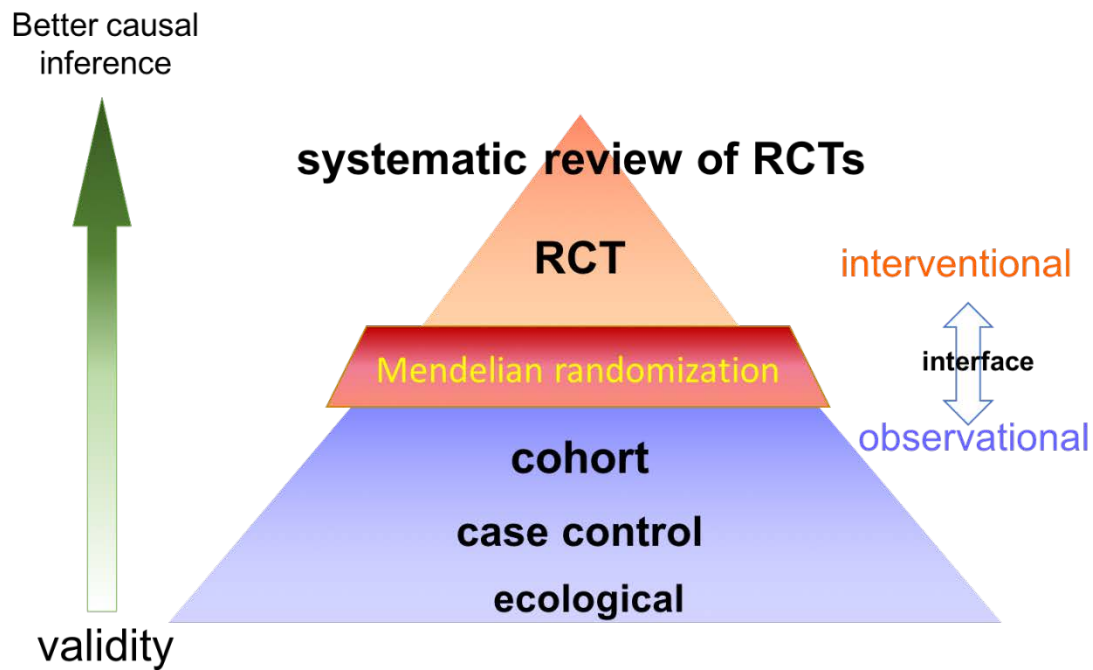


Figure 2. Metabolism of alcohol. Alcohol is metabolized primarily in the liver by the hepatic enzymes alcohol dehydrogenase (ADH1B) and acetaldehyde dehydrogenase (ALDH2). Acetaldehyde is an intermediate metabolite on the pathway that results in unpleasant symptoms including flushing, headache and nausea. Genetic variants causing increased activity of ADH1B (e.g. *ADH1B*2*) or reduced activity of ALDH2 (e.g. *ALDH2*2*) result in increased concentrations of acetaldehyde, which, due to the unpleasant effects, tends to result in a lower alcohol consumption. Because genetic variants are inherited on average independently from other genetic and environmental factors, they can be used in Mendelian randomization analyses to make causal inference on the relationship between alcohol and risk of disease.

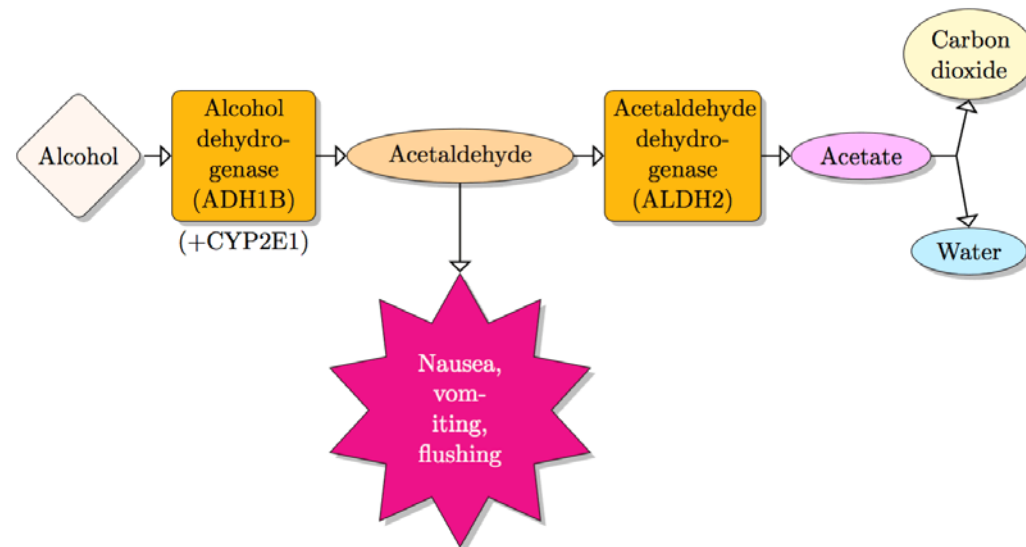


Figure 3. Using a phenotype as a proxy for genotype in MR analysis – directed acyclic diagram showing how the genotype-specific phenotype can be used as an instrumental variable to estimate the causal association between exposure and outcome.

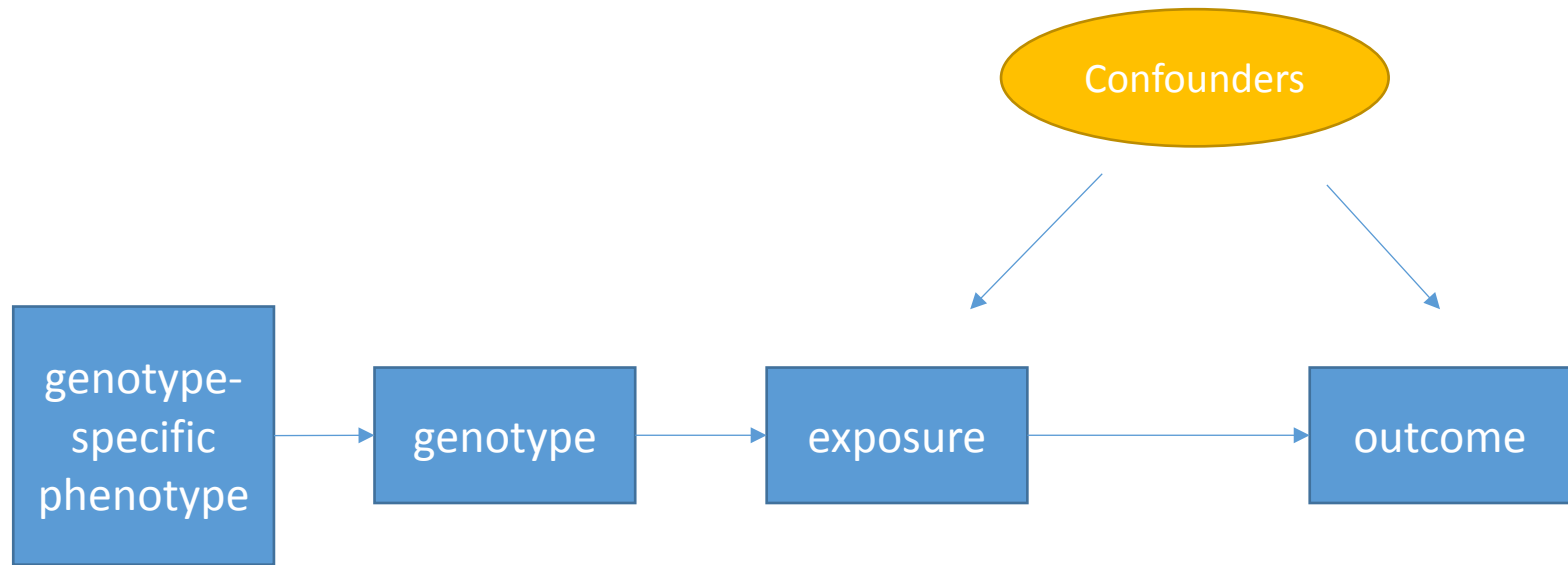


Table 1. Relationship between *ALDH2* genotype, alcohol flushing, and distribution of ethanol consumption and acetaldehyde levels.

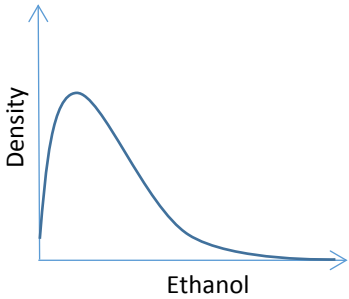
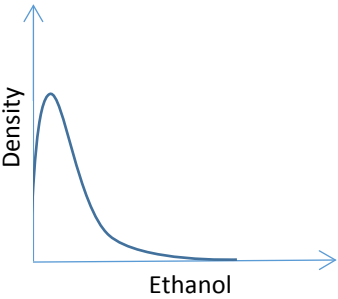
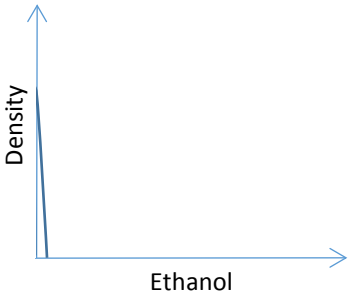
Trait	<i>ALDH2</i> genotype			Effect
	*1/*1	*1/*2	*2/*2	
Alcohol Flushing	No	Yes	Yes	Dominant
Ethanol consumption				Additive
Acetaldehyde levels	+	+++ (if drinks normally) +++++ (if drinks a lot)	Minimal (if non-drinker) +++++ (if light drinker; very rare)	Interaction with ethanol

Table 2. Suitability of selected instrumental variables for assessing causal relationships between alcohol and examples of chronic disease outcomes

Instrumental variable	Outcome/ likely causal component	Confounding	Pros/Cons	Causal inference	Public Health Relevance
None (conventional analysis using self-reported alcohol)	Cancer (acetaldehyde)	+++	Pro: can be conducted in any study with self-reported alcohol and disease outcomes Con: massive potential for residual confounding that limits interpretation	Potentially unreliable	Informative
	CVD (alcohol)	+++++		Extremely unreliable (not suitable for causal inference; suitable for generation of hypothesis)	Not informative (J-shape relationships add to societal ‘confirmation bias’ that alcohol may be beneficial)
Flushing as proxy for alcohol metabolism genotype (limited to Asian populations)	Cancer (acetaldehyde)	+ *	Pro: no genotype needed Cons: limited to East Asians; only suitable for diseases where causal component is alcohol and not acetaldehyde (see Table 1)	Limited (non-dominant; see Table 1)	Informative
	CVD (alcohol)			Possible assuming valid instrument	
Measured alcohol metabolism genotype	Cancer (acetaldehyde)	None ϕ	Pro: most reliable for causal inference (in absence of RCT data) Cons: <i>ALDH2</i> *2 is limited to East Asian populations, strong instrument; while <i>ADH1B</i> is polymorphic worldwide, it is a weaker instrument	Valid	Very informative – closest approximation to data from RCT
	CVD (alcohol)				

Legend: * confounding may arise if flushing response is non-specific to genotype; ϕ assuming assumptions of MR are satisfied

