

Mendelian Randomisation and Causal Inference in Observational Epidemiology

Nuala A. Sheehan*, Vanessa Didelez, Paul R. Burton, Martin D. Tobin

The Problem of Inferring Causality in Epidemiology

The notion of risk is central to epidemiological research, both in its original context of studying conditions thought to be caused by a particular factor and, more broadly, in predicting the probability of a condition for prognostic purposes. For prognostic research, all factors associated with the outcome are of interest, whether they are causal or not. In aetiological research, on the other hand, causality is meaningful. Here, the focus is often on assessing the effect of some modifiable exposure on a disease with a view to informing health interventions at the individual or population level, or health advice for particular risk groups. For such intervention or advice to be effective, it is important to verify that the observed association between the exposure and disease means that the exposure is in fact causal for the disease. For example, once the relationship between periconceptual maternal folate supplementation and risk of neural tube defects was established [1,2], the United States, Canada, and Chile implemented mandatory fortification of cereal flour and related foods with folic acid and reported reductions in neural tube defect incidence between 27% and just over 50% [3]. However, observational research has had several high-profile failures when exposures that seemed to affect disease risk were later shown to be non-causal in follow-up randomised controlled trials (RCTs). For instance, observational evidence that seemed to suggest that vitamin E is protective for cardiovascular disease, beta-carotene for cancer, and, more recently, oestrogen for dementia, has now been refuted [4]. Since only candidate causes with the strongest observational

Research in Translation discusses health interventions in the context of translation from basic to clinical research, or from clinical evidence to practice.

Five Key Papers in the Field

Chen et al., 2008 [9] A recent application of the method that combines information from several studies and uses a genetic variant as a proxy for an exposure that is difficult to measure.

Hernán and Robins, 2006 [8] A recent overview of what can and what cannot be done in epidemiological studies with instrumental variables.

Davey Smith et al., 2005 [5] A comment on the wider picture of where genetic epidemiology can contribute to public health research.

Davey Smith and Ebrahim, 2003 [7] The first main paper detailing the relevance of the method to epidemiological research and providing many examples.

Katan, 1986 [10] This briefly outlines the original idea behind the method of Mendelian randomisation as it is commonly used now.

support tend to be followed up in RCTs when these are possible, it is likely that many more reported observational findings are not actually causal [5].

Inferring causality from observational data is problematic as it is not always clear which of two associated variables is the cause and which the effect, or whether both are common effects of a third unobserved variable, or confounder (see Glossary). The direction of causality can sometimes be determined by temporal criteria (e.g., the cause must precede the effect) or from knowledge of the underlying biology. Confounding is more difficult to deal with because it is mainly due to social, behavioural, or physiological factors that are difficult to measure and control for. In practice, one can never be sure that the relevant confounders have been identified and accounted for. Besides the fact that RCTs are not feasible or ethical for many exposures of public health relevance, such as toxins, physical activity, or complex nutritional regimes, observational

studies also have some advantages over RCTs; for example, the subjects in the latter are not always representative of the population for which an intervention is being considered [6]. “Mendelian randomisation” provides an alternative way of dealing with the problems of observational studies [6–9], especially for the case where confounding is believed to be present but cannot be controlled for because it is not fully understood.

Mendelian Randomisation

We outline the idea now known as “Mendelian randomisation” using the example provided by Katan

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Abbreviations: ApoE, apolipoprotein E; CHD, coronary heart disease; CI, confidence interval; RCT, randomised controlled trial

Nuala A. Sheehan, Paul R. Burton, and Martin D. Tobin are in the Department of Health Sciences, University of Leicester, Leicester, United Kingdom. Vanessa Didelez is in the Department of Mathematics, University of Bristol, Bristol, United Kingdom.

* To whom correspondence should be addressed. E-mail: nas11@le.ac.uk

[10] in his early description of the concept in 1986, although the first implementation of this basic idea in an epidemiological setting under the flag of “Mendelian randomisation” was more recent [11]. Details of the derivation of the approach and its nomenclature are provided in a recent review [12].

In the mid-1980s, there was considerable debate over the hypothesis that low serum cholesterol levels might directly increase the risk of cancer. Alternative explanations for the observed association were that cholesterol levels were lowered by the presence of latent tumours in future cancer patients (reverse causation), or that both cancer risk and cholesterol levels might be affected by confounding factors like diet and smoking. The observation that individuals with abetalipoproteinaemia, and hence negligible levels of serum cholesterol, did not seem to be predisposed to cancer led Katan to the idea of finding a larger group of individuals genetically inclined towards lower cholesterol levels. The apolipoprotein E (*ApoE*) gene was known to affect serum cholesterol, the *ApoE2* variant being associated with lower levels. Katan’s idea was that many individuals will carry the *ApoE2* variant and thus will naturally have lower cholesterol levels from birth. Crucially, since genes are randomly assigned during meiosis (which gives rise to the name “Mendelian randomisation”), these *ApoE2* carriers will not be systematically different from carriers of the other *ApoE* alleles in any other respect, and in consequence there should be no confounding. Only if low serum cholesterol is really causal for the disease should cancer patients have more *ApoE2* alleles than controls. Otherwise the distribution of *ApoE* alleles should be similar in both groups. This can be easily checked from the observed distributions.

Katan’s reasoning corresponds exactly to what is known as an instrumental variable method in econometrics [13–16]. The genetic variant acts as a so-called instrumental variable (or instrument) and helps to disentangle the confounded causal relationship between intermediate phenotype and disease. Once this theoretical connection had been made, epidemiologists were able to learn from

and adapt the methods that were so well known in econometrics [7,17].

The three key assumptions for Katan’s idea to work, and hence for a genetic variant to qualify as an instrumental variable, are illustrated graphically in Figure 1 and interpreted as follows.

1. The genetic variant is unrelated to (independent of) the typical confounding factors, i.e., the graph has no arrow (in either direction) connecting *ApoE* with the confounders.
2. The genetic variant is (reliably) associated with the exposure, i.e., there *is* an arrow connecting *ApoE* to serum cholesterol and we can accurately quantify the relationship this represents.
3. For known exposure status (cholesterol level) and known confounders (if the confounders were observable), i.e., *conditional* on exposure and confounders, the genetic variant is independent of the outcome, i.e., *ApoE* does not provide any additional information for the prediction of cancer once these two variables are measured. An equivalent way of expressing this, which is less precise but perhaps more intuitive, is to say that there is no direct effect of genotype on disease (no single arrow between *ApoE* and cancer) nor any other mediated effect other than through the exposure of interest (no other routes in the graph between *ApoE* and cancer).

Note that these assumptions have to be justified from background knowledge of the underlying biology. Neither the first nor the third assumption can be tested statistically since they depend on the confounding factors, which, by definition, are unobserved. The first assumption means that you must have reasonable belief that your genetic variant is unaffected by the sort of confounding that might generally be expected of such an exposure–disease relationship. Fortunately, the very basis of Mendelian randomisation rests on the knowledge that alleles are randomly assigned from parental alleles at meiosis (see above), and this implies that, across the population, genetic effects are relatively robust, although not immune to confounding [7,18]. Furthermore, the

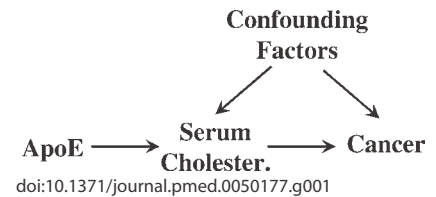


Figure 1. The *ApoE* Genotype as an Instrumental Variable in a Mendelian Randomisation Application

The arrows can be thought of as representing causal relationships, but this is not what matters here. What is essential is the *absence* of an arrow between *ApoE* and the confounders and between *ApoE* and cancer, as detailed in the three key assumptions in the text.

type of information needed to explore this assumption is often available in practice, as it is usually well-studied genetic variants that are proposed as instruments. Assumption 3 demands a comprehensive understanding of the underlying biological and clinical science, and may appropriately be considered in a sensitivity analysis. Unlike the first and third, the second assumption *can* formally be tested using the observed data, and the method works better the stronger the association between gene and exposure.

If the three assumptions seem reasonable (i.e., Figure 1 is believable), then it can be shown that, as Katan originally hypothesised, a simple statistical test of association between the *ApoE* genotype and cancer amounts to a test for causal effect of cholesterol levels on cancer [19].

The idea of using a gene as an instrument to test for a causal effect of an intermediate phenotype on a disease has been used for a range of other traits, some of which are summarised in Table 1 [9,20–28]. For example, raised plasma fibrinogen levels have been associated with an elevated risk of coronary heart disease (CHD) in large-scale prospective studies, prompting suggestions that methods to reduce fibrinogen levels should be sought [29]. If the fibrinogen–CHD relationship were causal, then such interventions could have considerable clinical and public health benefits. However, interventions to lower plasma fibrinogen levels would not be warranted if the association was explained by confounding or reverse causation. Doubts about a causal link between fibrinogen and CHD have

Table 1. Examples of Mendelian Randomisation Studies

Disease or Outcome	Exposure or Phenotype of Interest	Genetic Variant (Instrument)	Findings	Reference(s)
Coronary heart disease	Fibrinogen	Beta-fibrinogen <i>G-455→A</i> and <i>C-148→T</i> polymorphisms	Evidence from these MR studies would suggest that reported observational plasma fibrinogen–CHD associations are explained by confounding or reverse causation.	[20,21]
Stroke	Homocysteine	<i>MTHFR C677T</i> polymorphism	MR evidence is consistent with a causal relation between homocysteine concentration and stroke.	[22]
Carotid intima media thickness	CRP	<i>CRP</i> gene (haplotypes derived from 5 SNPs)	MR evidence from this study does not support a causal role for CRP in the development of a thickened intima media (and potentially later CHD).	[23]
Myocardial infarction	CRP	<i>CRP</i> gene +1444 <i>C>T</i> polymorphism	MR evidence from this study does not support a causal role for CRP in non-fatal myocardial infarction.	[24]
Metabolic phenotypes	CRP	<i>CRP</i> gene +1444 <i>C>T</i> polymorphism	CRP has been associated with metabolic phenotypes in observational studies, but MR evidence from this study does not support a causal relationship between CRP levels and any of the metabolic phenotypes studied.	[25]
Blood pressure and hypertension	CRP	<i>CRP</i> gene 1059 <i>G/C</i> polymorphism	Evidence from this study does not support a causal relationship between CRP levels and blood pressure or hypertension.	[26]
Blood pressure	Alcohol intake	<i>ALDH2</i> *2 allele	MR evidence supports the hypothesis that (even modest) alcohol intake increases blood pressure.	[9]
Type 2 diabetes	MIF	<i>MIF</i> gene (4 SNPs)	MR evidence supports a causal role for MIF in the development of T2D in women.	[27]
Fat mass	Maternal BMI	<i>FTO</i> gene <i>rs9939609</i> polymorphism	MR evidence does not support the hypothesis that maternal BMI during pregnancy affects fat mass in children aged 9–11 years.	[34]
Physical function in 65- to 80-year-olds	IL-18	Four <i>IL-18</i> gene polymorphisms	MR evidence supports the hypothesis that high IL-18 levels are a cause rather than a consequence of disability in the elderly.	[28]

This table gives a range of examples that illustrate how Mendelian randomisation is used in practice. It is not intended to give a complete and balanced overview of the area, however, as there are many more studies that are not referred to here. BMI, body mass index; CRP, C-reactive protein; IL, interleukin; MIF, macrophage migration inhibitory factor; MR, Mendelian randomisation; SNP, single nucleotide polymorphisms; T2D, type 2 diabetes.
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been raised by evidence that the association is considerably attenuated by adjustment for smoking, body mass index, and plasma apolipoprotein B/A₁ ratio [20], and that there are many known correlates of fibrinogen, only some of which are typically measured and adjusted for in individual studies [30]. Furthermore, bezafibrate was found to reduce plasma fibrinogen in randomised controlled trials, but it did not have a greater effect on CHD risk than could already be explained by its cholesterol-lowering effect [31].

Additional light can be cast on this relationship from relevant genetic studies. A recent large meta-analysis of genetic association studies of fibrinogen promoter region polymorphisms (*G-455→A* and *C-148→T*) showed that there was a mean increase in fibrinogen of 0.12 g/l (95% confidence interval [CI] 0.09 to 0.14) per copy of the *A* or *T* allele. However, these same alleles were *not* associated with CHD risk: the odds ratio per allele was 0.98 (95%

CI 0.92 to 1.04) [21]. Since the 95% confidence interval includes the null hypothesis value of 1, we cannot reject the null hypothesis at the 5% level and hence conclude that the data provide little or no evidence for a causal effect of fibrinogen on CHD. This could be due to random error or lack of power of the statistical test, which is a problem with genetic association studies when relatively small effects are being sought. The findings are also consistent with the hypothesis that the associations shown previously in observational studies are partially or wholly explained by reverse causation or confounding. Of course, as with any test, the fact that an exposure *appears* to be non-causal does *not* necessarily mean that it is not clinically useful. Clearly, it would be dangerous to stop investigating the role of fibrinogen in CHD risk because of such an outcome. What *is* implied, however, is that more investigation is required before making any great investment in intervening on fibrinogen levels.

Mendelian randomisation can also be applied when the exposure of interest is a modifiable behaviour rather than an intermediate phenotype. For example, Chen et al. [9] consider the causal effect of alcohol intake on blood pressure. An RCT would be problematic here, and measurement of alcohol intake is prone to error. Hence, observational data have to be considered in a setting where the causal relationship of interest is known to be heavily confounded. In some populations, a particular variant (*2) of the *ALDH2* gene is quite common. The *2 variant is associated with accumulation of acetaldehyde, and therefore unpleasant symptoms, after drinking alcohol. Carriers of this variant tend to limit their alcohol consumption, and alleles at the *ALDH2* locus can hence be used as a surrogate or proxy for alcohol intake [9]. Based on this assumption, a Mendelian randomisation meta-analysis approach, combining evidence from several studies, indicated that

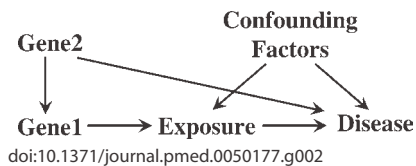


Figure 2. A Mendelian Randomisation Study Where the Chosen Instrument Is in Linkage Disequilibrium with a Variant Associated With, or Causal For, the Disease. Note that the direction of the arrow depicting the statistical association between the two genes is interchangeable.

previous observational evidence on the beneficial effects of moderate drinking on blood pressure were possibly misleading. Exploring biological complexity is another important application of the method, although we have not focused on this aspect here. Li et al. [32] use a Mendelian randomisation approach to infer parts of biological causal pathways, for example.

Problems and Limitations

The limitations of Mendelian randomisation fall into two main categories. Firstly, the key assumptions for a genotype to be an instrument (see above) may not be plausible, in which case any inference about the causal effect will typically be biased. Such limitations include the presence of linkage disequilibrium, genetic heterogeneity, pleiotropy, population stratification, canalisation, or lack of knowledge about the confounding factors. These limitations have received a lot of attention in the literature [6,7,33]. However, graphs can be used as a visual check, and some apparent violations may not actually be problems in practice [19].

For example, Figure 2 addresses the case where the chosen instrument, *Gene1*, is in linkage disequilibrium with another gene, *Gene2*, which has not been observed. Here, *Gene2* directly affects the disease level or risk, and hence *Gene1* is not an instrument

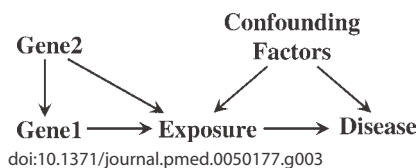


Figure 3. A Mendelian Randomisation Study Where the Chosen Instrument Is in Linkage Disequilibrium with a Variant That Is Also Causal for the Intermediate Exposure

due to violation of the third key assumption. However, if *Gene2* only affects the disease via its effect on the same intermediate exposure, as shown in Figure 3, there is no such violation and *Gene1* can be used as an instrument in a Mendelian randomisation analysis. Note that *Gene1* would also qualify as an instrument if its association with the exposure was *only* via its association with *Gene2* (Figure 4). Hence, it does not really matter whether *Gene1* or *Gene2* is the causal variant for the exposure when they are in linkage disequilibrium, as either one qualifies as an instrumental variable in this case.

A similar check for violations can be applied to the situation described in Lawlor et al. [34], where the hypothesised causal effect of maternal adiposity on offspring adiposity is investigated using maternal *FTO* genotype as an instrument. The reason that one must also adjust for offspring *FTO* genotype in the relevant regression in order to perform a Mendelian randomisation analysis can be illustrated quite simply by the graph in Figure 5. Without adjusting for (conditioning on) offspring *FTO*, key assumption 3 would be violated due to the existence of an alternative path to the outcome via this genotype. Note that this situation is specific to the graph in Figure 5, which assumes that there is no other confounder of offspring *FTO* and offspring adiposity (such as paternal *FTO*).

If the three key assumptions of an instrumental variable are satisfied by the genetic variant, testing for a causal effect of phenotype on disease by testing for an association between genotype and disease is straightforward for most practical purposes. Any statistical test that is appropriate for the variables being considered will suffice. However, *calculation* of the magnitude of the causal effect requires additional strong assumptions, such as linearity of all relationships (e.g., constant increase of disease with exposure) and no interactions. If these assumptions are satisfied, we can obtain an estimate of the causal effect from a mathematically simple combination of the observed genotype–disease and genotype–exposure associations [13]. The second class of limitations of Mendelian randomisation concerns the validity of such additional assumptions. These limitations have not generated

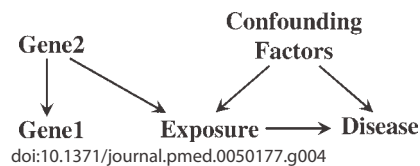


Figure 4. A Mendelian Randomisation Study Where the Chosen Instrument Is Not Directly Causal for the Exposure, But Is in Linkage Disequilibrium with Another Variant That Is

so much discussion to date, although in many observational studies the outcome is a binary variable, and, under the mathematical models that are typically applied—e.g., logistic or probit regression—conventional linearity is not satisfied [19]. In consequence, the estimate that is valid in the all-linear case should not really be applied to binary outcome data, although it has sometimes been advocated [17,26]. Generalisations of the instrumental variable method to the non-linear case can be found in the literature [8,15,35–39], but are typically aimed at very different kinds of applications. Their usefulness in the context of Mendelian randomisation has yet to be investigated. It is, perhaps, important to stress that these extra distributional assumptions are only an issue for estimation of the magnitude of the causal effect and not for testing for the presence of such an effect.

The Future for Mendelian Randomisation

A Mendelian randomisation analysis does not aim to identify genetic factors that are causal for disease risk in order to target individuals on the basis of their genotype. On the contrary, the focus is on the causal association between an exposure and a disease with a view to informing the potential impact of non-genetic interventions on that exposure. To that end, such analysis exploits a well-studied genetic

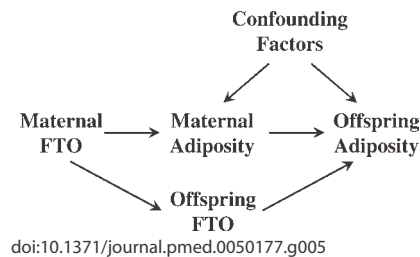


Figure 5. Maternal *FTO* Only Qualifies as an Instrument Conditional On Offspring *FTO*

factor for its *known* relationship with the exposure.

In order to widen the applicability of the approach, more general methods for the common, but statistically non-standard, case with a binary disease outcome need to be developed.

In particular, the relevance to observational epidemiology of related methods in other areas, especially in terms of the particular assumptions required, is currently being investigated. We should also stress the importance of obtaining good estimates from genetic association studies, in particular ensuring sufficiently large sample sizes with adequate power to detect the typically modest effects one might expect for the determinants of common multifactorial diseases [6,20,40]. The need to formally combine information from different sources, such as the large biobanks that are currently being set up worldwide, is also essential [41].

Mendelian randomisation has received its fair share of criticism (e.g., [42]). One objection is that good genetic instruments are not easy to find, but recent rapid advances in genetic epidemiology are addressing this issue [5]. Most criticisms concern the violations of the key assumptions implicit in Figure 1. Confounding of the genotype–disease relationship is one such violation that has received some attention. However, it has recently been re-emphasised that this violation may not be as serious as may at first appear because, as outlined above, Mendelian randomisation analyses are fundamentally less susceptible to confounding than conventional epidemiology analyses [18].

Summary

It is often unavoidable (and sometimes desirable) to use observational data to infer causality, but it may then be difficult to disentangle causation from association, especially in the presence of confounding. We would argue that some of the confusion and misleading interpretations of results from observational studies are partly due to the lack of a clear formal approach to distinguish between association and causation. Causal terminology is often used loosely in the medical literature. It is intended to convey more than a simple association between potential

Glossary

Genetic Terms

Alleles are the different variants of a gene at a locus. They are sometimes called polymorphisms.

Canalisation is a developmental compensation that can atone for disruptive environmental or genetic forces.

Genetic heterogeneity refers to the situation where a phenotype is influenced by several alleles, usually at different genetic loci.

Linkage disequilibrium refers to (statistical) association between alleles at different loci. One reason for such an association is that the relevant genetic loci are physically close on the chromosome, and the alleles tend to be inherited together.

Pleiotropy refers to the situation where a genetic variant has more than one specific phenotypic effect.

Population stratification occurs when allele frequencies and disease rates, or allele frequencies and exposure rates, vary widely between different sub-

risk factors and their effects, but this is rarely made explicit. More formal approaches are based on the idea of a hypothetical intervention [43,44], which seems particularly suited to the present context where we have potential health interventions in mind. These formal approaches highlight the usefulness of Mendelian randomisation studies for inferring causality and enable precise specification of the key assumptions (as depicted in Figure 1) necessary for the method to be valid.

Given the tendency of high-profile findings to persist in the literature, and influence public health and clinical policy, long after they have been formally refuted by RCT analyses [4], and given the expense and the scientific and ethical constraints of RCTs, it is fortunate that advances in biology, biotechnology, and epidemiology have provided us with an alternative tool, in the shape of Mendelian randomisation, that can help us to formally assess causality based on observational data. But the approach demands a sound understanding both of the underlying biomedicine and of the statistical

groups of the population and cause an association between the two at the overall population level.

Statistical Terms

Conditional independence: For variables X , Y , and Z , we say that X and Y are *conditionally independent given* Z if knowledge of X (or alternatively Y) does not improve our prediction of Y (alternatively X) once we actually know Z .

Confounding: The effect of a variable X on another variable Y is said to be *confounded* if the observed association between X and Y does not correspond to the causal effect. Confounding is often due to the existence of another cause of Y that is also associated with X .

Interaction: Variables X_1 and X_2 *interact* in their association with Y if the association of X_1 with Y varies for different values or levels of X_2 .

Linear relationship: The relationship between variables X and Y is *linear* if the change in Y caused by a unit change in X is constant for all values or levels of X . Any departure from this criterion is a *non-linear* relationship.

assumptions invoked in its application. If it is used wisely, Mendelian randomisation could make a major contribution to our understanding of the aetiological architecture of complex diseases; but if it is used unthinkingly, it could sow seeds of confusion and set back progress in bioscience. This short article is aimed at encouraging the former and avoiding the latter. ■

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