# Mendelian randomization: genetic anchors for causal inference in epidemiological studies

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**Abstract**

Observational epidemiological studies are prone to confounding, reverse causation and various biases, and have generated findings that have proved to be unreliable indicators of the causal effects of modifiable exposures on disease outcomes. Mendelian randomization is a method that utilises genetic variants that are robustly associated with such modifiable exposures to generate more reliable evidence regarding which interventions would produce health benefits. The approach is being widely applied, and various ways to strengthen inferences given the known potential limitations of Mendelian randomization are now available. The integration of genetic information into population-based epidemiological studies presents translational opportunities that capitalise on the investment in genomic discovery research.

Many examples exist of apparently robust observational associations between behavioural, pharmacological or physiological measures and disease risk which, when subjected to randomised controlled trials, do not deliver the anticipated health benefits because the causal effect has been incorrectly inferred. These include many nutritional factors (e.g. several vitamins), pharmacological agents (e.g. hormone replacement therapy) and circulating biomarkers (e.g. HDL cholesterol). Confounding, reverse causation and various biases can generate the associations, and even with careful study design and statistical adjustment incorrect causal inference is possible.[[1]](#endnote-1) [[2]](#endnote-2) The recognition of these problematic aspects of epidemiological investigation has led to the application of a series of methods aimed at improving causal inference[[3]](#endnote-3) [[4]](#endnote-4). A successful approach is to use genetic variants as indicators of exposure that are not subject to the influences that vitiate conventional study designs, an approach known as Mendelian randomization (MR)[[5]](#endnote-5) [[6]](#endnote-6). We will not repeat the many detailed reviews that now exist of MR5 [[7]](#endnote-7) [[8]](#endnote-8) [[9]](#endnote-9) [[10]](#endnote-10) [[11]](#endnote-11) [[12]](#endnote-12) , nor summarise the hundreds of empirical studies applying the technique to a wide range of exposures and disease outcomes, rather, after a brief summary of the foundational principles, we will outline recent developments and potential future directions of the field.

## Basic principles of Mendelian randomisation

Inferring the causal direction between correlated variables is a pervasive issue in biology that simple regression analysis cannot answer. The association between two variables could reflect a causal relationship, but the direction of causality (e.g. A causing B or B causing A) is not clear. Furthermore there maybe unobserved factors that influence both variables and lead to their association (confounding) (Figure 1). In the latter scenario the effect of the independent variable on the outcome may be zero. Even if the hypothesised causal direction were correctly specified, if the independent variable is correlated with some unobserved or imprecisely measured confounders then the estimate of its causal effect could be biased. Mendelian randomisation (MR) is a technique aimed at unbiased estimation of the existence and magnitude causal effects

Suppose that trait A and trait B are correlated, it follows that if this correlation arises because A is causing B, then any variable that influences trait A should also influence trait B. The key to inferring a causal relationship between A and B is to identify an ‘instrument’ that is reliably associated with A. Biologists are in a privileged position in this regard because virtually all traits of interest are at least partially influenced by genetic effects, and genetic effects can serve as excellent instruments for a number of reasons. First, and most crucially, in a genetic association the direction of causation is from the genetic polymorphism to the trait of interest, and not vice versa. Second, conventionally measured environmental exposures are often associated with a wide range of behavioural, social, and physiological factors that confound associations with outcomes[[13]](#endnote-13). Genetic variants, on the other hand, can serve as unconfounded indicators of particular trait values. Third, genetic variants and their effects are subject to relatively little measurement error or bias. Fourth, the actual causal variant for the trait is not required, a marker in linkage disequilibrium (LD) with the causal variant will satisfy the conditions for MR. Finally, in the era of genome wide association studies (GWAS) and high throughput genomic technologies, genetic instruments are routinely available on large well-phenotyped studies .

### Analogy between Mendelian randomization and randomised controlled trials

An intuitive way to understand how MR can be used to infer causality is by analogy with randomised controlled trials (RCTs). In RCTs the study participants are randomly allocated to one or another treatment, thus any potential confounding between treatment and outcome is avoided, and causal inference is unambiguous. MR creates a similar scenario for us. Supposing a particular allele is robustly related to trait A, and trait A causes trait B. Alleles are largely passed from parents to offspring independent of environment, and subjects who inherit the allele are in effect being assigned a higher dosage of trait A, while those who don’t inherit the allele are assigned a lower dosage. Empirical evidence that there is lack of confounding of genetic variants with factors that confound exposures in conventional observational epidemiological studies is extensive13 [[14]](#endnote-14), though it is important to take measures to avoid introducing confounding through population stratification (Table 2). As in randomized controlled trials, groups defined by genotype will experience an on-average difference in exposure to trait A, whilst not differing with respect to confounding factors. A by-genotype analysis is equivalent to an intention-to-treat analysis in a randomized controlled trial. We outline the conditions for MR and how it is performed in Box 1.

To date MR has been successfully applied to a wide range of observational associations, covering applications to the causal effects of biomarkers on disease, understanding the correlation between physiological measures, estimating the causal effects of various behaviours and specifying maternal intrauterine influences (Table 1). Though there are a number of limitations to MR that should be considered when using this approach (Table 2), they have been discussed at length elsewhere5 [[15]](#endnote-15) [[16]](#endnote-16) [[17]](#endnote-17) [[18]](#endnote-18) [[19]](#endnote-19) [[20]](#endnote-20). The remainder of this paper will outline recent developments in MR that seeks to address some of these limitations.

## Recent extensions to basic Mendelian Randomization

### *Use of multiple variants to increase power and test assumptions*

Ideally MR is performed using a single variant whose biological effect on the trait for which it is an instrument is understood. However this comes with two potential limitations, which can be partially mitigated by increasing the number variants used as instruments.

First, the genetic effect may not be particularly large, resulting in a weak instrument. By increasing the number of variants the proportion of variance explained by the instrument increases, thus improving precision in 2SLS[[21]](#endnote-21). Combining these into a weighted allele scores is generally the optimal approach in this context[[22]](#endnote-22).

Second, the variant could be pleiotropic or in LD with a variant that affects the outcome, thus violating conditions (2) or (3) for being a valid instrument. One can interrogate this potential caveat by using multiple instruments. For example it would be increasingly improbably that two, three, or more independent instruments all result in the same conclusion due to perfectly balancing pleiotropic effects on both traits. For a convincing example demonstrating the causal influence of low-density lipoprotein cholesterol (LDL-C) on coronary heart disease (CHD) see Figure 2, where 9 polymorphisms from 6 genes independently lead to very similar predicted causal effects using instrumental variables analyses[[23]](#endnote-23).

Typically genetic variants are only used as instruments if they are reliably detected and replicated in GWAS. But predictive power is often improved when SNPs that do not reach significance thresholds are also included, the rationale being that there will be false negatives due to small effect size[[24]](#endnote-24). Using this approach can improve the power of MR, but caution should be made due to the increased chance of including pleiotropic effects (Box 2).[[25]](#endnote-25)

### *Two sample Mendelian randomization*

It is often the case that an observational association between two variables exists, but high measurement costs or lack of appropriate biospecimens leads to relatively small datasets with both measurements and instruments. Methods have been developed to perform IV analysis when the exposure variable and the outcome variable are measured in two independent datasets[[26]](#endnote-26),and these can be applied in the MR context[[27]](#endnote-27).

Another scenario in which two sample MR can be used is if the data in which MR is being performed is the same as is being used to identify instruments. GWAS is known to lead to overestimation of genetic effect sizes due to the phenomenon of winner’s curse, and this can lead to bias in MR. Subsetting the data into two (or more) samples for estimation and testing can mitigate this problem. This method has been applied in a study that demonstrates that childhood adiposity leads to a reduction in physical activity[[28]](#endnote-28).

### *Bidirectional Mendelian randomization*

A major limitation of the MR is that using a single genetic variant it is difficult to distinguish between an exposure causing an outcome and an outcome causing a trait, because the genetic variant could have its primary influence on either variable. For example, atheroma and BMI influence C-reactive protein (CRP) levels, and apparent misleading causal effects can be generated if a genetic variant primarily influencing atheroma or BMI is mistaken as being a variant for CRP[[29]](#endnote-29).

Aside from focusing on instruments for which there exists some degree of biological understanding, another method is to use bi-directional MR. Here, instruments are required for both variables and MR is performed in both directions. If trait A causes trait B, then the instrument, will be associated with both A and B. However, a second instrument specific to trait B, , will be associated with trait B, and not with trait A. This method has been used to demonstrate that BMI influences CRP levels, rather than vice versa[[30]](#endnote-30) [[31]](#endnote-31) and that obesity influences vitamin D levels, rather than vice versa.[[32]](#endnote-32).

### *Factorial Mendelian randomization*

The manner by which causes of disease act together to increase disease risk can have important public health implications, as above additive effects lead to the clustering of risk factors generating a greater burden of disease in the population. For example evidence exists that the combined influence of obesity and heavy alcohol consumption on the risk of liver disease is greater than multiplicative[[33]](#endnote-33). It is difficult to estimate such effects, however, as confounding can be magnified when examining two already confounded risk factors. Factorial randomised controlled trials overcome this issue by randomising each treatment independently, allowing characterisation of interactions between them[[34]](#endnote-34). Likewise, combinations of genetic variants can be used to perform factorial MR studies to obtain unconfounded estimates of the effect of co-occurrence of the two risk factors for disease.

### *Complexity of association and multiphenotype Mendelian randomization*

Often a particular genetic variant may lead to an intermediate phenotype whose biological function may not be reliable captured when indirectly measured. For example, antioxidants are thought to lower risk of coronary heart disease (CHD), so increasing circulating levels of extracellular superoxide dismutase (EC-SOD, a scavenger of superoxide anions) might be hypothesised to decrease CHD risk. However, MR studies have shown that genetic variants associated with higher circulating EC-SOD actually increase CHD risk.[[35]](#endnote-35) An explanation for this apparent paradox is that circulating levels of EC-SOD may actually increase only when they are moving away from arterial walls, thus the *in situ* anti-oxidative activity is reduced. In order for MR to be used effectively in mediation, a clear biological interpretation of the relationship between intermediate phenotypes and disease outcomes is important.

In some situations genetic variants tend to be associated with multiple intermediate phenotype, and estimating the causal effect of one particular intermediate phenotype is problematic. For example, many categories of blood lipids such as various fractions of HDL cholesterol and triglycerides are observationally associated with coronary heart disease, but they are also highly (inversely) correlated, and observational studies cannot reliably separate their effects.[[36]](#endnote-36). Many of the genetic variants related HDL-C and triglycerides, of which there are many, associate with both measures.[[37]](#endnote-37) Whereas factorial MR can be applied to multi-phenotype relationships when the genetic effects influence each phenotype distinctly, in this example this is not possible because constructing an instrument that purely relates to one phenotype is problematic. This is an example of a demonstrable case of type I pleiotropy (Box 2). A first step towards this problem is to use regression methods to attempt to separate the effects, and two independent studies utilizing this approach have recently suggested that the causal influence of triglycerides was robust, whereas the apparent protective effect of HDL-C was not[[38]](#endnote-38) [[39]](#endnote-39). Both the appropriateness of different statistical approaches and whether reliable answers can be obtained in the multiphenotype context remain areas of active investigation.

### *Hypothesis free Mendelian randomization*

The majority of MR studies have been focused on testing hypotheses that arose from associations between traits seen in observational studies. But is this only the tip of the iceberg? An illustrative example of there being vastly more potential associations than those already known was presented by Blair et al, who after mining the medical records of 110 million patients uncovered 2909 associations between Mendelian diseases and complex traits, the majority of which were previously unreported[[40]](#endnote-40). As high-throughput ‘omics technologies continue to reduce in price, datasets with comprehensive genotyping and phenotyping are destined to grow, and in principle it should be possible to construct instruments for many exposures and through data mining obtain evidence regarding outcomes caused by these exposures19**.** More speculatively, generation of instruments from within the data and performance of split-sample or jackknife IV analysis could allow resolution of causal direction within networks of phenotypes without advance specification of which exposure or outcome is being examined[[41]](#endnote-41)**.**

## Conclusions

Resolving observational correlations into causal relationships is an elusive problem at the heart of biological understanding, pharmaceutical development, prevention of disease and medical practice. MR is a statistically robust method that can support this endeavour, and its scope for application widens as the cost of data generation continues to reduce.

## Acknowledgements

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Table 1: Examples of Mendelian randomisation

|  |  |  |  |
| --- | --- | --- | --- |
| Type | Exposure / trait | Disease / outcome | Conclusion |
| Biomarkers | CRP | Coronary heart disease | Observational association between CRP and coronary heart disease is a result of confounding and/or reverse causation[[42]](#endnote-42) |
|  | Serum Iron | Parkinson’s Disease | Higher serum iron levels lower the risk of Parkinson’s Disease[[43]](#endnote-43) |
|  | Uric acid | Coronary heart disease | Observational association between uric acid and coronary heart disease is due to confounding by BMI[[44]](#endnote-44) |
|  | Macrophage migration inhibitory factor (MIF) | Type 2 diabetes | Elevated MIF, instrumented by a genetic marker, is associated with higher risk of type 2 diabetes, suggesting a causal role[[45]](#endnote-45) |
|  | Interleukin 6 (IL6) | Coronary heart disease | IL6 increases the risk of coronary heart disease [[46]](#endnote-46) [[47]](#endnote-47) |
| Behaviours | Smoking | Anxiety/depression | Anxiety and depression amongst smokers does not appear to be a consequence of smoking[[48]](#endnote-48) [[49]](#endnote-49) |
|  | Alcohol consumption | Blood pressure | Alcohol use increases blood pressure[[50]](#endnote-50) |
| Physiological measures | BMI | Symptomatic gallstone disease | Three independent variants associated with BMI are associated with elevated risk of symptomatic gallstone disease, suggesting a causal role for BMI[[51]](#endnote-51) |
| Maternal influences (corrected for genetic correlation with child) | Alcohol consumption | Childhood School Performance | The observational association finding that moderate maternal alcohol intake is associated with more favourable school performance is due to confounding, and the casual association is in the opposite direction[[52]](#endnote-52) |
|  | Maternal BMI | Fat mass | Fat mass in children aged 9-11 is not is not strongly influenced by BMI of mothers during pregnancy[[53]](#endnote-53) |

Table 2: Limitations of Mendelian randomisation

|  |  |  |
| --- | --- | --- |
| Limitation | Role in Mendelian randomization (MR) studies | Approaches to evaluating or avoiding the limitation |
| Low statistical power | MR studies are often of low power and effect estimates are imprecise because of this | Increase sample size and/or combine genetic variants so they explain more of the variance of the intermediate phenotype |
| Reverse causation | A genetic variant may be causing the disease outcome which in turn causes the biomarker, or the causal direction could be in the opposite direction. 2SLS will not distinguish between these cases | Bi-directional MR can be used to distinguish between the two causal models. |
| Population stratification | Spurious associations used as instruments can lead to faulty causal inference | Restrict analyses to ethnically homogeneous groups, families and apply correction methods using ancestrally informative markers or principal components from genome wide data |
| Reintroduced confounding though pleiotropy | A genetic variant may directly influence more than one post-transcriptional process. Known to be the case for some genetic variants | When possible utilise cis variants with respect to the intermediate phenotype under study, as these may be less likely to have pleiotropic effects. Apply multiple instrument approaches with more than one independent genetic variant as unlikely pleiotropy will generate the same associations for different instruments |
| Linkage disequilibrium (LD) induced confounding | LD is crucial in genetic association studies as it allows marker SNPs to proxy for un-genotyped causal SNPs. However this can reintroduce confounding if LD leads to the association of SNPs related to more than one post-transcriptional process. This case will be similar to the pleiotropy situation | Studies can be carried out in populations with different LD structures. Approaches to avoiding distortion by pleiotropy will also counter problems due to LD |
| Canalization / developmental compensation | During development compensatory processes may be generated that counter the phenotypic perturbation consequent on the genetic variant utilized as an instrument | No general approach developed, although context–specific biological knowledge can be appealed to. Period of lifecourse when influence of genetic variation on IPs emerge can indicate whether canalization could, in principle, be an issue |
| Lack of genetic variants to proxy for modifiable exposure of interest | No reliable genetic variant associations for many intermediate phenotypes of interest, although an increasing number of these now identified | Continued genome wide and sequencing based studies |
| Complexity of associations | Without adequate biological knowledge misleading inferences regarding intermediate phenotypes and disease may be drawn | Increased biological understanding of genotype – phenotype links |

Figure 1: Directed acyclic graph (DAG) depicting MR

A. MR can be used to test the hypothesis that trait A causes trait B, provided that conditions (1), (2), and (3) are met adequately, governing that is a valid instrument. B. In bi-directional MR prior knowledge of the causal direction between traits A and B (if any) can be elucidated if valid instruments are present for each trait.

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Figure 2: Effect of lower LDL-C on risk of CHD (taken from Ference *et al.* (2012))

Boxes represent the proportion risk reduction (1-OR) of CHD for each exposure allele plotted against the absolute magnitude of lower LDL-C associated with that allele (measured in mg/dl). Vertical lines represent 1 SE above and below the point estimate of proportional risk reduction. SNPs are plotted in order of increasing absolute magnitude of associations with lower LDL-C. The line (forced to pass through the origin) represents the increase in proportional risk reduction of CHD per unit lower long-term exposure to LDL-C.

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Box 1: Performing Mendelian randomisation

Conventional instrumental variable (IV) analysis requires that the instruments are valid, and in order to be valid they must meet three conditions. An instrument for trait A must be

1. reliably associated with trait A;
2. associated with the outcome (trait B) only through trait A; and
3. independent of unobserved confounders that influence traits A and B after conditioning on observed confounders.

In MR, condition (1) is straightforward to test, but (2) and (3) cannot be directly proven. For example, if the variant is pleiotropic (see Box 2), or if it is in linkage disequilibrium (LD) with a genetic variant that influences the outcome through a different mechanism, this can lead to erroneous causal estimation. If the above conditions are met, then the unbiased estimate of the effect of trait A on the outcome, B, can be made using two-stage least squares (2SLS) regression.

In stage 1 a predictor for A is constructed from its instrument, and in stage 2 the effect of the predictor for A acting on B is estimated. The intuition here is that A is potentially associated with B due to many confounding effects, and we wish to estimate the effect of A on B that occurs only via the instrument, which we know is in the correct causal direction for A. Thus, if the predictor for A is associated with B in the estimate from stage 2 then this is only occurring through a path which has no confounding. There exist several software implementations for performing various instrumental variable analyses, including 2SLS (e.g. the “ivregress” package in STATA; the “systemfit” package in R[[54]](#endnote-54)); three stage least squares regression (e.g. the “systemfit” package in R[[55]](#endnote-55)); the general case of IV estimation using the generalised method of moments (“gmm” package in R[[56]](#endnote-56)), and STATA routines for running subsample and two sample IV estimation.27

Box 2: Consequences of pleiotropy on the interpretation of MR

Pleiotropy is the phenomenon by which a single locus influences multiple phenotypes[[57]](#endnote-57). Depending on the form it takes, pleiotropy is a potential limitation to interpretation of MR, so distinguishing between its different types is important. In the context of MR there are two mechanisms by which pleiotropy occurs: a single process leading to a cascade of events (e.g. a locus influences one particular protein product, and this causes perturbations in many other phenotypes); or a single locus directly influencing multiple phenotypes[[58]](#endnote-58) [[59]](#endnote-59). Amongst its many names, the former has been termed “spurious pleiotropy” [[60]](#endnote-60), or “type II pleiotropy” [[61]](#endnote-61); the latter “biological pleiotropy” [[62]](#endnote-62)or “type I pleiotropy” [[63]](#endnote-63). Type II pleiotropy is not only unproblematic for Mendelian randomization, it is the very essence of the approach, in which the downstream effects of a perturbed phenotype are estimated through the use of genetic variants that relate to this phenotype. Thus the instrument of common variation in *FTO*, known to influence body mass index (BMI)[[64]](#endnote-64), probably through influencing caloric intake[[65]](#endnote-65) [[66]](#endnote-66) is associated with a wide range of downstream phenotypes blood pressure and hypertension[[67]](#endnote-67), coronary heart disease [[68]](#endnote-68), fasting insulin, glucose, HDL cholesterol and trigylcerides[[69]](#endnote-69), bone mineral density[[70]](#endnote-70), chronic renal disease [[71]](#endnote-71), and diabetes[[72]](#endnote-72). These associations are expected, as higher BMI influences these traits, and it would be an error to consider them “pleiotropic” effects of *FTO* variation that vitiate MR investigations.

Type I pleiotropy, however, is problematic for the interpretation of MR. Estimates of the degree of pleiotropy suggest that type II pleiotropy is the more pervasive form[[73]](#endnote-73), with type I pleiotropy being more pronounced at the level of the gene than at the level of single SNPs[[74]](#endnote-74). Greater pleiotropic effects are seen for mutations with larger effects on the primary trait[[75]](#endnote-75) [[76]](#endnote-76), as would be anticipated for type II pleiotropic influences that are downstream effects of considerable perturbation of the primary trait.

Ultimately, guarding against potentially erroneous causal inference due to type I pleiotropy can be achieved by restricting instruments to well-powered genetic effects which plausibly act directly on the trait (e.g. the instrument for CRP levels is located within the promoter region of the *CRP* gene). It is tempting (and often necessary) to use genetic profile scores, gaining statistical power by using multiple instruments, but this does come at the cost of reduced clarity in interpretation of the results.

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