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EDITORIAL

# On the use of Mendelian randomization to infer causality in observational epidemiology

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**This editorial refers to ‘Lifetime body mass index and later atherosclerosis risk in young adults: examining causal links using Mendelian randomization in the Cardiovascular Risk in Young Finns study’<sup>†</sup> by M. Kivimäki et al., on page 2552**

For obvious ethical reasons, risk factors cannot be assessed in randomized controlled trials. Epidemiologists therefore usually identify risk factors using observational data. It is, however, difficult to establish causal relationships between risk factors and common complex diseases because observational studies are prone to spurious results due to confounding factors, reverse causation, and/or selection biases.<sup>1</sup> Atherosclerosis is a common complex trait influenced by several cardiovascular risk factors that tend to cluster. In this context, determining whether a putative risk factor is causally related to atherosclerosis, independently of all other risk factors (i.e. *ceteris paribus*), is a challenging task.

## What is Mendelian randomization?

The concept of Mendelian randomization refers to the random allocation of alleles at the time of gamete formation. By analogy with the fact that the random allocation of treatment in a randomized controlled trial renders confounding unlikely, a genetic variant of interest should not be associated with known and unknown confounding factors.<sup>2</sup> Mendelian randomization has recently been proposed as a new tool to overcome some of the problems encountered in observational epidemiology, such as reducing residual confounding and protecting against reverse causation.<sup>3</sup> In Mendelian randomization, a functional genetic variant, or a variant in strong linkage disequilibrium with it, is used to retrieve an unbiased estimate of the association of a modifiable exposure [e.g. C-reactive protein (CRP), fibrinogen, or body mass index (BMI)] with a disease (e.g. coronary heart disease, stroke, or atherosclerosis).<sup>1</sup> As such, Mendelian randomization may prove a valuable tool to infer causality in cardiovascular observational epidemiology. However, it is not, and should not be viewed, as a panacea, and its limitations should be clearly acknowledged. The concept of Mendelian randomization is an application of the

theory of instrumental variables.<sup>2,4,5</sup> Instrumental variables are used to make causal inference in non-experimental conditions and have been widely explored by econometricians.<sup>2</sup> As illustrated in Figure 1 of the paper by Kivimäki et al.,<sup>6</sup> an instrumental variable (e.g. rs9939609 *FTO* variant) is a variable that is associated with the outcome (e.g. atherosclerosis) only through its association with the exposure of interest (e.g. lifetime BMI). The reader should keep in mind that the use of instrumental variables in statistical genetics is still in its infancy and that more theoretical work is needed in this context. An important limitation in statistical genetics is the weakness of the instrument, i.e. the genetic variant is only weakly correlated with the exposure of interest.

## The limitations of Mendelian randomization

Mendelian randomization studies represent a special case of genetic association studies and therefore also suffer from similar limitations. So far, the findings of genetic association studies in the field of complex traits, including cardiovascular traits, have been difficult to replicate. Several reasons may explain these difficulties. First, any single genetic variant only explains a small proportion of the trait variance, so that very large sample sizes are needed to achieve a reasonable power. Secondly, it is not easy to identify relevant functional genetic variants, so that most associations are indirect and rest on linkage disequilibrium, which may vary from one population to the other. Thirdly, complex diseases are probably influenced by numerous gene–gene and gene–environment interactions, which again may differ from one population to the other. Fourthly, the level of genetic heterogeneity underlying complex cardiovascular traits is still unclear. Most genetic association studies rely on the assumption that the ‘common disease, common variant hypothesis’ holds, i.e. the same variants are causal in affected individuals, which is uncertain.

In addition to the general limitations cited above, the Mendelian randomization approach in observational epidemiology suffers from other limitations, some of which are more specific to this approach:<sup>7–9</sup> (i) a suitable genetic variant to study the exposure

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of interest does not always exist; (ii) the association between selected genetic variants and gene product (or associated trait) is often not very strong (i.e. the instrument is weak with correlations between genetic variant and gene product usually being  $<0.05$ ); (iii) there may be confounding by population stratification; (iv) there may be confounding by linkage disequilibrium; (v) pleiotropy may exist (i.e. the gene of interest influences many phenotypes); (vi) the genetic variant of interest may affect selective survival, before or after birth, so that the genotypic frequencies at entry into the study do not match those obtained after meiosis; (vii) there may be segregation distortion at the locus of interest; (viii) there may be canalization and developmental compensation (i.e. a functional adaptation to a specific genotype may influence the expected genotype–disease association); and (ix) a parent-of-origin effect may exist.

## The present study in perspective

The paper by Kivimäki *et al.*<sup>6</sup> describes the effect of lifetime BMI on carotid intima-media thickness (CIMT) and various atherosclerotic risk factors in young adults in Finland using both conventional ordinary least square regression and instrumental variable two-stage least square regression approaches. The results suggest that increased BMI during childhood is associated with increased risk of atherosclerosis in early adulthood. In this study, previous phenotypic data from a large and long follow-up study are available. In contrast, genetic data only consist of one single nucleotide polymorphism (SNP) located within the *FTO* gene. Note, however that, in this study, genotyping additional *FTO* SNPs and inferring haplotypes did not improve the strength of the observed signal, which suggests that the measured *FTO* SNP and estimated haplotypes are probably detecting the same signal.

Kivimäki *et al.* have used an original, yet risky, approach in that they used Mendelian randomization with an exposure (i.e. lifetime BMI) that is not the direct gene product, unlike some previous studies on the *CRP* gene, CRP levels, and various outcomes.<sup>10–13</sup> Why is this approach risky? First, it is risky because the instrument (i.e. the genetic variant) is very weak, as illustrated by the low proportion of BMI variance (in this particular case 0.4%) explained by the rs9939609 *FTO* variant. As a consequence, the estimated effect size becomes very imprecise: for instance, although the effect size obtained for the association of lifetime BMI on adult CIMT using the instrumental variable approach was substantially higher than the one obtained using ordinary least square regression, the confidence interval was much larger and the result not statistically significant. Secondly, it is risky because, in such a situation, it is difficult to ensure that the gene (e.g. *FTO*) effect on the outcome of interest (e.g. CIMT) only acts via the exposure of interest (e.g. lifetime BMI). Failure to satisfy this latter condition could lead to biased estimates of effect size.

Despite these potential limitations, the example appears to be well chosen: (i) the association between this *FTO* genetic variant and BMI has been repeatedly observed in independent studies; (ii) the association of the *FTO* gene with other phenotypes susceptible to influence carotid atherosclerosis (e.g. diabetes mellitus) appears to occur via BMI, so that the assumptions underlying Mendelian randomization should not be violated in this particular case. This study nicely illustrates how two approaches

(conventional ordinary least square regression and instrumental variable two-stage least square regression), which suffer from different limitations, may complement each other. If one is willing to accept that the assumptions underlying the instrumental variable approach are met, the results based on Mendelian randomization suggest that the association between lifetime BMI and CIMT is causal. It will be of great interest to see if these results can be confirmed in other large prospective cohort studies, in particular in other ethnic groups. The importance of these findings lies in the potential for early prevention of atherosclerosis via BMI reduction.

Although it is as yet unclear to what extent Mendelian randomization studies can and will improve causal inference in observational epidemiology, this potential is certainly worth exploring further. The study by Kivimäki *et al.*<sup>6</sup> illustrates how Mendelian randomization can be used to increase our confidence that a specific association, i.e. lifetime BMI and CIMT, is causal using observational data. These results suggest that genetic epidemiology may reinforce the findings of observational epidemiology.

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