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Commentary: Orienting causal relationships between two phenotypes using bi-directional Mendelian randomization

Rebecca C Richmond[1] and George Davey Smith[1]

[1]MRC Integrative Epidemiology Unit (IEU), Population Health Sciences, Bristol Medical School, University of Bristol, Oakfield House, Oakfield Grove, Bristol, BS8 2BN, UK

The need for bi-directional Mendelian randomization

Where the causal relationship between two related phenotypes is unknown, bi-directional Mendelian randomization can be used to orient the causal direction(s) of effect using two independent sets of genetic variants related to each of the phenotypes. In this volume of the IJE, Xu and colleagues investigate the causal direction of the effect between asthma and adiposity using bi-directional Mendelian randomization (MR) (1). In the study, the authors find evidence in support of a causal effect of body mass index (BMI) on asthma, but conclude that "the effect of asthma on body mass index is small, if present at all". These conclusions were drawn from the results of both two-sample and one-sample Mendelian randomization analysis using summary statistics from a large genome-wide association study (GWAS) of BMI (GIANT) (2), a large GWAS of asthma (TAGC) (3), as well as GWAS of BMI and asthma conducted using data from UK Biobank (4).

The study represents the first bi-directional MR analysis of BMI and asthma in adults, although previous MR studies have assessed the uni-directional effect of BMI on asthma in both children and adults (5-7) and one previous study has performed bi-directional MR in childhood, albeit in a relatively small one-sample analysis (n~2500) (8). It also contributes to a growing collection of bi-directional MR studies, which require some considerations to be made (in addition to those in conventional MR studies) in order to orient and establish the causal relationships. Some of these considerations are outlined in this commentary.

Orienting the causal direction of effect

Bi-directional MR relies of four key variables: trait A, trait B, a genetic instrument for trait A (Z_A) and a genetic instrument for B (Z_B), which may be used to determine the existence and direction(s) of causality between the two traits (Figure 1).

Figure 1

<<Figure 1 here>>

The scenario which is most easily determined using bi-directional Mendelian randomization is a), where Z_A and Z_B can be used to establish that there is no causal relationship between A and B in either direction. In scenario b), where trait A causes trait B and not vice-versa, then Z_A will be associated with both A and B whereas Z_B will be associated with trait B and not with trait A. In scenario c), where trait B causes trait A and not vice-versa, then Z_B will be associated with both B and A whereas Z_A will be associated with trait A and not with trait B. In scenario d), where trait A causes trait B and trait A causes trait B, then Z_A will be associated with both A and B and Z_B will be associated with both trait A and B.

However, scenarios b) – d) may be incorrectly inferred if the core MR assumptions are violated. Of particular threat is violation of the exclusion restriction assumption, which is "no association independent of the exposure". This may occur if a genetic variant is related to both trait A and trait B but is used to instrument the incorrect trait (A or B), which may occur when there is a limited biological understanding of the variant. For example, if trait A influences trait B, then a GWAS with

adequate statistical power will identify a genetic variant with its primary influence on trait A as being associated with trait B. This will lead to spurious conclusions if this variant is then used as an instrument for B (Z_B). This problem was initially raised in the context of deriving instruments from genetic variants which do not surpass strict thresholds for "genome-wide significance", and thus picking up variants primarily associated with other traits which can "contaminate" the genetic instrument (9). However, as GWAS increase in sample size and power, the chances of identifying genetic variants primarily associated with trait A that associate with trait B at genome-wide significance increase, and including those SNPs in the MR analysis to assess the causal effect of trait B on trait A will potentially lead to erroneous inferences of causality (10).

Improving correct identification of causal directionality

One way to alleviate this problem is to ensure that the two instruments (Z_A and Z_B) are not marginally associated with each other. In this study, the authors report a manual search of various online resources (GWAS Catalog, Ensembl and Phenoscanner) to identify whether the genetic variants being used in each instrument have been previously associated with the other trait (or phenotypes with a potential causal path to the other trait). No instruments used in the two-sample analysis were found in relation to the other trait using these online resources. However, in the one-sample MR analysis in UK Biobank, the authors note that 75/74,107 SNPs associated with BMI at $p<5x10^{-8}$ before linkage disequilibrium (LD) pruning were also associated with asthma at the same threshold, while 234/12,900 SNPs associated with asthma before LD pruning were also associated with BMI at the same threshold. These SNPs were subsequently excluded from the MR analysis.

While it is optimal to ensure that the two genetic instruments being used in bi-directional MR are independent, one issue with SNP pruning based on associations with other traits is that these associations might reflect vertical rather than horizontal pleiotropy (i.e. they have an entirely indirect effect on Trait B via Trait A), and therefore removing these SNPs from the genetic instrument will increase the risk of type II error. The authors also report use of heterogeneity-based outlier adjustment methods (including penalized weighted median and MR-PRESSO) to further eliminate horizontal pleiotropic paths between the genetic instruments and the other trait. Again, while applying these sensitivity analyses is advocated, it should also be highlighted that these approaches typically reduce the standard error of a causal effect estimate after removing those SNPs which deviate from the majority, which can increase the risk of type I error (11).

Another approach which wasn't used in this current study, but which is advocated in the context of bi-directional MR is the MR Steiger method (12). This has been developed to determine outliers based on whether they are likely to act first through trait A or trait B. Taking scenario b) in Figure 1, as the correlation of the variant Z_A with trait B is a product of both the variant Z_A -trait A correlation and the causal effect of trait A on trait B, the variant Z_A -trait A correlation should be greater than the variant Z_A -trait B correlation under a model of vertical pleiotropy (where variant Z_A influences trait A which in turn influences trait B). Steiger filtering removes those SNPs from the genetic instrument, Z_A, which have a stronger correlation with the trait B than trait A (11). In a previous bi-directional MR study of education and intelligence, MR Steiger showed that many of the education SNPs explained more variance in intelligence than educational attainment, and when filtering was done the estimated causal effect of years of schooling on intelligence was reduced by half (13). MR Steiger has been integrated into the TwoSampleMR R package (11) to assess both continuous and binary traits (estimated on the logit liability scale) and so could have been used in this instance to assess validity of the SNPs being used to instrument both BMI and asthma.

While the use of MR Steiger in the context of bi-directional MR is an important component that improves correct identification of causal directionality, it is important to bear in mind that it can produce erroneous results under some levels of differential measurement error and unmeasured confounding (12). Large differences in sample sizes between the exposure and outcome GWAS may also impact the efficacy of this approach. As such, a series of sensitivity analyses should be carried out to evaluate the impact of a range of possible sample sizes, measurement error values and confounding effects (12). In addition, recently developed methods, including latent causal variable (LCV) (14) and Bayesian network analysis (BNA) (15), can also provide insights into bi-directionality when genetic instruments are available as causal anchors for both traits, potentially with greater power. Both of these approaches rely on assumptions that are different to those of bi-directional MR with Steiger filtering, and the focus should be on evaluating the overall body of evidence from different approaches, rather than considering there to be a single "correct" approach (16).

Establishing evidence for a causal effect in both directions

To appraise evidence of causality in a bi-directional MR, we would want to maximise statistical power to detect the causal effect in both directions. In order to make a direct comparison, we would require that both traits A and B have equally strong genetic instruments, similar sample sizes and similarity in the variable type (i.e. continuous, binary, categorical) (17, 18).

In this particular study, the authors state that the genetic instrument for BMI (composed of 75 SNPs) explains 1.55% of the variation in BMI, while the asthma genetic instrument (composed of 8 SNPs) explains approximately 0.3% of the variation in asthma. Given that these instruments have both been applied in a two-sample approach where the second sample is UK Biobank (with roughly equivalent sample size in both studies), this suggests higher power to detect a causal effect of BMI on asthma than vice versa. However, it is also important to bear in mind that the sample size required for a given level of power is greater with a binary outcome (i.e. asthma) than a continuous outcome (i.e. body mass index) (18). As formal power calculations have not been presented, it is difficult to determine whether power was equivalent for determining causal effects. In addition, while it appears that the one-sample MR improved power to detect causal effects, the strength of the genetic instruments used in this analysis was not clearly presented.

Interpreting causal effect estimates in both directions

As well as asserting evidence of causality in both directions in a bi-directional MR, it is also of interest to determine the prevailing direction of effect if a bi-directional relationship is shown to be present. In this study, the authors claim that the magnitude of the protective effect of asthma on BMI is much smaller than adverse effect of BMI on asthma. This is difficult to directly assert based on the data which are presented on different scales i.e. the odds of asthma per SD unit increase in BMI versus change in BMI z-score per 1-log unit increase in asthma risk. Furthermore, when interpreting causal estimates with binary exposures using Mendelian randomization, there are a number of considerations and assumptions which need to be made (19), which include:

- Whether there is monotonicity of the genetic effect on the exposure
- Whether there is homogeneity of the causal effect of the exposure on the outcome
- Whether the binary exposure is a dichotomization of an underlying continuous risk factor

As such, it is often simpler to report on the existence (rather than the magnitude) of the causal effect when assessing a binary exposure, which makes establishing a prevailing direction of causality in bi-directional MR difficult. Nonetheless, if these assumptions can be made, there are options for causal estimation with a binary exposure which allow estimates to be converted onto a more

clinically meaningful scale. For example, the point estimate of a -0.004 change in BMI z-score per 1-log unit increase in risk of asthma obtained from the two-sample MR fixed-effects inverse variance weighted (IVW) meta-analysis could be converted to represent, for example a doubling in odds of asthma risk (i.e. by multiplying the effect by ln(2)) (19). This would equate to an 0.003 z-score reduction in BMI, which with an SD of 4.785 is equivalent to a 0.014 kg/m^2. While this is indeed a small effect, the authors could have made more of an attempt to contextualise this, as was done for the point estimate obtained from the fixed-effect IVW for the effect of BMI on asthma (OR 1.18 per SD unit increase in BMI), which was compared with the effect obtained for BMI on both type 2 diabetes (OR 2.8) and cardiovascular disease (OR 1.53) in the same two-sample framework.

Another consideration should be made when conducting two-sample MR to interpret a causal effect estimate for a binary exposure e.g. presence or absence of the disease. The estimate of the effect of the binary exposure cannot be attributed to the exposure itself in such situations. This is obvious when two-sample MR studies are carried out in outcome samples that contain only a small number of participants who have experienced the exposure in question. For example, two-sample MR studies treating schizophrenia as the exposure carried out in UK Biobank as the outcome sample are doing so in a setting where a very small percentage of the study population have experienced the disease, and it would be misleading to interpret the effects as being those of schizophrenia itself. Indeed, it is possible to conduct a two-sample MR where the outcome dataset includes no individuals who have experienced the binary exposure (e.g. disease). In these situations it is clear that such analyses would reveal effects that cannot be interpreted as those of the exposure on the outcome. In such situations the causal effect estimates should be interpreted as reflecting the effects of the genetic liability to the exposure (20). Whilst this interpretation is obvious when the exposure is a disease which is not seen in the outcome population, the same principles may apply in many other cases in which the liability to the binary exposure can influence the outcome directly. Thus even in this particular study aiming to establish the causal effect of asthma on body mass index in two-sample MR, with asthma being a relatively common in the outcome GWAS (~11.6% in UK Biobank) (1), it is uncertain whether the findings reflect the effect of asthma itself, or of liability to asthma (without asthma having necessarily arisen), on the outcomes. Indeed, conducting MR analyses in a population without the disease in question (e.g. among children when studying the effects of late-onset diseases) is one way to demonstrate liability effects that cannot be due to the disease itself. With the use of individual-level data (with recorded and dated information on exposure and outcome) it would be possible to estimate the downstream causal effects of developing the disease, as opposed to the effects of the liability to the disease (20).

Triangulating findings

As mentioned, several pleiotropy-robust sensitivity analyses as well as one-sample MR have been performed in order to triangulate findings with regards to the bi-directional effects (1). Whereas effect estimates were generally consistent for the effect of BMI on risk of asthma, hence warranting the emphasise on this direction of causality, it is worth noting that the point estimates for the protective effect of asthma on BMI varied and were typically of a greater magnitude in several of the pleiotropy-robust sensitivity analyses as well as in the one-sample MR study conducted in the UK Biobank. While these approaches in turn make different assumptions and have various limitations, many of which are highlighted in the discussion, these findings should not be entirely downplayed. In particular, any evidence of a difference between main IVW analysis and pleiotropy-robust methods could indicate the presence of horizontal pleiotropy, which in this instance may be diluting the reverse causal effect in the IVW approach. Additional attempts are advocated which conceptually synthesize the findings with those of other studies using different designs and with predicted orthogonal biases, to allow for strengthened causal inference (16, 21).

Conclusions

The study by Xu and colleagues has performed both one- and two-sample MR using large study sizes to determine the direction of causation between body mass index and asthma in adulthood (1). The authors emphasise the consistent evidence for an adverse effect of BMI on asthma risk, although the possibility of a possible negative-feedback effect of BMI on asthma could not be completely discounted. While the authors were understandably reluctant to discuss the pathophysiological mechanisms underlying the observed effects, findings of an adverse effect of obesity on asthma are supported by existing evidence from both observational and Mendelian randomization studies. The small protective effect of asthma on BMI is indeed a counterintuitive finding which should not be completely dismissed, rather further work to determine whether this is a true causal effect is required. Furthermore, several additional steps, including recent extensions to Mendelian randomization and other complementary approaches, could be taken to better orient, establish and interpret directions of causality both within the context of BMI and asthma and other plausible bi-directional relationships.

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Conflict of interest

None to declare

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