Bias correction for inverse variance weighting Mendelian randomization

Ninon Mounier[1,2] **Zoltán Kutalik**[1,2,3,*]

Abstract

Background

Inverse-variance weighted two-sample Mendelian randomization (IVW-MR) is the most widely used approach that utilizes genome-wide association studies (GWAS) summary statistics to infer the existence and the strength of the causal effect between an exposure and an outcome. Estimates from this approach can be subject to different biases due to the use of weak instruments and winner's curse, which can change as a function of the overlap between the exposure and outcome samples.

Methods

We developed a method (`MRlap`) that simultaneously considers weak instrument bias and winner's curse, while accounting for potential sample overlap. Assuming spike-and-slab genomic architecture and leveraging LD-score regression and other techniques, we could analytically derive, reliably estimate, and hence correct for the bias of IVW-MR using association summary statistics only.

Results

We tested our approach using simulated data for a wide range of realistic settings. In all the explored scenarios, our correction reduced the bias, in some situations by as much as 30 folds. When applied to real data on obesity-related exposures, we observed significant differences between IVW-based and corrected effects, both for non-overlapping and fully overlapping samples. Additionally, our results are consistent with the fact that the strength of the biases will decrease as the sample size increases. Finally, we also showed that the overall bias is also dependent on the genetic architecture of the exposure, and traits with low heritability and/or high polygenicity are more strongly affected.

Conclusions

Our method not only reduces bias in causal effect estimation but also enables the use of much larger GWAS sample sizes, by allowing for potentially overlapping samples.

Key words: Mendelian randomization, Weak instrument bias, Winner's curse, Sample overlap

[1]University Center for Primary Care and Public Health, University of Lausanne, 1010, Switzerland

[2]Swiss Institute of Bioinformatics, Lausanne, 1015, Switzerland

[3]Department of Computational Biology, University of Lausanne, Lausanne, 1015, Switzerland

[*]Correspondence should be addressed to zoltan.kutalik@unil.ch

1 Introduction

Mendelian randomization (MR) is a method that uses genetic variants (typically single nucleotide polymorphisms, SNPs) as instrumental variables (IVs) to infer the existence and the strength of the causal effect between an exposure and an outcome[1]. In particular, two-sample summary data MR[2], that requires solely genome-wide association study (GWAS) summary statistics has become increasingly popular. The reason for this is that in the last decade, GWASs have drastically increased in sample size[3]. The resulting summary statistics are often publicly available allowing not only the identification of genetic variants independently associated with a particular exposure, i.e. IVs, but also the look up of the variants' effects on the outcome trait in different samples. Each IV provide an independent estimate for the causal effect and these estimates can then be combined using an inverse variance-weighting (IVW) approach[2]. MR relies on three main assumptions (Figure 1): (1) Relevance – IVs must be robustly associated with the exposure. (2) Exchangeability – IVs must not be associated with any confounder of the exposure-outcome relationship. (3) Exclusion restriction – IVs must be independent of the outcome conditional on the exposure and all confounders of the exposure-outcome relationship.

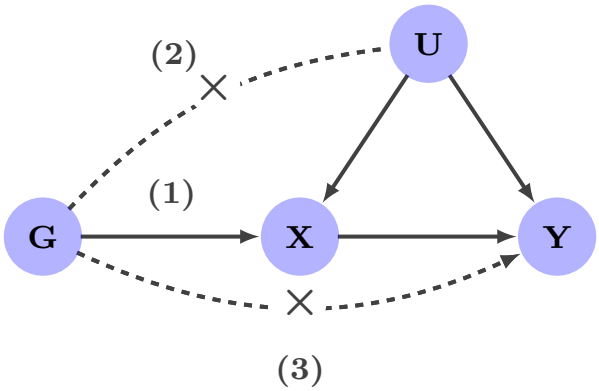

Figure 1: Main assumptions of Mendelian randomization
(1) Relevance – instrumental variables (IVs), denoted by G, are strongly associated with the exposure. (2) Exchangeability – G is not associated with any confounder of the exposure-outcome relationship. (3) Exclusion restriction – G is independent of the outcome conditional on the exposure and all confounders of the exposure-outcome relationship (i.e. the only path between the IVs and the outcome is via the exposure).

Additionally, two-sample MR methods expect the exposure and the outcome GWAS summary statistics to be obtained from independent samples. Sample overlap acts as a modifier for two well-known sources of bias in MR, weak instrument bias and winner's curse. Two-sample MR estimates obtained from non-overlapping samples are known to be biased towards the null. When using overlapping samples, the causal effect estimate can be biased towards the observational correlation, which includes the correlation induced by confounders[4]. Most MR methods assume that SNP-exposure effects are measured without noise (NO Measurement Error, NOME assumption)[5]. This simplification leads to regression-dilution bias, which increases as the instruments get weaker. For this reason, the bias introduced by the NOME assumption is referred to as weak instrument bias and it becomes more and more severe as the average variance of

the exposure explained by the IVs decreases[6]. When combined with sample overlap, the effect of weak instrument bias will move towards the observational correlation[7,8]. Using IVs strongly associated with the exposure and/or increasing the sample size can mitigate weak instrument bias[7]. Although the exact multiplicative bias due to the NOME assumption can be expressed analytically (proportional to the inverse of the F-statistic), the estimator for the multiplicative constant has typically high variance and works poorly in practice[5]. The simulation-extrapolation based SIMEX method proved to yield more robust corrections both for IVW[9] and MR-Egger estimates[5]. In addition, MR estimates are subject to winner's curse, which occurs when the same sample is used to select IVs and estimate their effect on the exposure. In such case, the observed IV effect on the exposure is not an unbiased estimator for its true effect and is likely to be overestimated (in absolute value). This would affect the causal effect estimate (underestimation in non-overlapping sample and bias towards the observational correlation in fully overlapping samples)[8]. Using a third independent sample to select instruments, and therefore avoid winner's curse, is not always possible. Based on the expectation of truncated normal distribution[10], a correction can be applied for the SNP-exposure effect sizes. However, the additional estimator variance such correction entails can outweigh the benefit of the reduced bias, which can be mitigated by directly maximising the conditional likelihood[11]. Still, all these methods account for winner's curse for a single SNP, but do not model the bias induced by the IV selection process from millions of potential markers, which is far more complex and depends on the underlying genetic architecture of the exposure. While previous approaches aimed at tackling one bias at a time, the intricate way these different sources of biases interplay with each other remains poorly understood, and there is currently no method that simultaneously tackles them. To fill this gap, we propose a new method called `MRlap`, which is a summary statistics-based MR framework that simultaneously takes into account weak instrument bias and winner's curse, while accounting for potential sample overlap and its effect as a modifier of these biases.

Another major source of bias in MR is pleiotropy. In the presence of uncorrelated pleiotropy (reducing the exclusion restriction to the InSIDE assumption[12]), the causal effect estimate from IVW-MR is still consistent. Correlated pleiotropy (which can be induced by the existence of a genetic confounder acting on both the exposure and the outcome), however, can lead to the violation of the second assumption and more severe biases. Some approaches can be used to relax this assumption by assuming that at least 50% of the instruments are valid[13] or that non-pleiotropic instruments are the most frequent[14]. Since our `MRlap` approach does not explicitly tackle pleiotropy, we compared the impact of different sources of biases (pleiotropy, weak instrument bias, winner's curse in the presence of potential sample overlap) on IVW-MR, pleiotropy-robust methods and `MRlap` results.

In this paper, we will first introduce a two-sample MR framework that simultaneously takes into account weak instrument bias and winner's curse, while accounting for potential sample overlap and its effect as a modifier of these biases, in order to obtain a corrected causal effect estimate. We will then test our approach and compare the proposed correction of the IVW-MR causal

effect estimate against its uncorrected counterpart (and some pleiotropy-robust methods) using a wide range of simulation settings, including scenarios with pleiotropy. Finally, to demonstrate its utility, we will apply our approach to obesity-related traits using UK Biobank[15] (UKBB) data.

2 Methods

2.1 Expectation of the causal effect estimate

Let X and Y denote two random variables representing two complex traits. Genotype data is denoted by G and its jth column by g_j (columns representing genetic variants, rows representing individuals). To simplify notation we assume that $E(X) = E(Y) = E(G) = 0$ and $Var(X) = Var(Y) = Var(G) = 1$. Let us assume that X is observed in sample A of sample size n_A, Y is observed in sample B of sample size n_B with an overlap of $n_{A\cap B}$ individuals between the two samples. The vector of realisations of Z^C is denoted by $\boldsymbol{z}^C$ for all variables ($Z = X, Y, G, g$) and samples ($C = A, B, A \cap B$). Let us assume the following models:

$$\begin{aligned} \boldsymbol{x}^A &= G^A \cdot \boldsymbol{\gamma_x} + \boldsymbol{\epsilon}_{\boldsymbol{x}}^A \\ \boldsymbol{x}^B &= G^B \cdot \boldsymbol{\gamma_x} + \boldsymbol{\epsilon}_{\boldsymbol{x}}^B \\ \boldsymbol{y}^B &= \alpha \cdot \boldsymbol{x}^B + G^B \cdot \boldsymbol{\gamma_y} + \boldsymbol{\epsilon}_{\boldsymbol{y}}^B \end{aligned} \tag{1}$$

where $\boldsymbol{\gamma_x}$ are the effect sizes of the genetic variants on X, $\boldsymbol{\gamma_y}$ are their pleiotropic effects on Y. Assuming that there is a single environmental confounder U acting linearly on both traits (as used for simulations) the error term can split into two parts : $\boldsymbol{\epsilon}_{\boldsymbol{x}}^C = \kappa_x \cdot \boldsymbol{u}^C + \boldsymbol{\varepsilon}^C$ and $\boldsymbol{\epsilon}_{\boldsymbol{y}}^C = \kappa_y \cdot \boldsymbol{u}^C + \boldsymbol{\varepsilon}^C$, where κ_x and κ_y refer to the effect of U on X and Y respectively, ε^C is independent of the confounder and C can take the values A, B or $A \cap B$ as above.
Under the INSIDE assumption[12] (INstrument Strength Independent of Direct Effect, i.e. horizontal pleiotropic effects are independent of the direct effect), $cov(\boldsymbol{\gamma_x}, \boldsymbol{\gamma_y}) = 0$ and $E[\boldsymbol{\gamma_y}] = 0$. We denote $\rho := cov(\boldsymbol{\epsilon_x}, \boldsymbol{\epsilon_y}) = \kappa_x \cdot \kappa_y$. It corresponds to the part of the observational correlation (r) due to a (non-genetic) confounder ($r = \rho + \alpha$). Note that genetic confounding as well as reverse causal effect are also affecting observational correlation, but as long as the instruments used for MR are not associated with the confounder nor the outcome, their effect would be captured by ρ.

The univariate effect size estimates from GWASs for genetic variant j are as follows:

$$\begin{aligned} (\widehat{\beta}_x^A)_j &= \frac{1}{n_A} \cdot (\boldsymbol{g}_j^A)' \cdot \boldsymbol{x}^A = \frac{1}{n_A} \cdot (\boldsymbol{g}_j^A)' \cdot \left(G^A \cdot \boldsymbol{\gamma_x} + \boldsymbol{\epsilon}_{\boldsymbol{x}}^A\right) \\ (\widehat{\beta}_y^B)_j &= \frac{1}{n_B} \cdot (\boldsymbol{g}_j^B)' \cdot \boldsymbol{y}^B = \frac{1}{n_B} \cdot (\boldsymbol{g}_j^B)' \cdot \left(\alpha \cdot \boldsymbol{x}^B + G^B \cdot \boldsymbol{\gamma_y} + \boldsymbol{\epsilon}_{\boldsymbol{y}}^B\right) \end{aligned} \tag{2}$$

where the genotype data for genetic variant j for individuals in sample A is denoted by $\boldsymbol{g}_j^A$.

We intend to use MR to estimate the causal effect of X on Y. We will use m linkage disequilibrium (LD)-independent genetic variants as IVs. Let us now consider the fixed-effect inverse-variance weighting meta-analysis for the ratio estimates for the causal effect α. Each IV

j provides a ratio estimate:

$$\widehat{\alpha}_j = \frac{(\widehat{\beta}_y^B)_j}{(\widehat{\beta}_x^A)_j} \tag{3}$$

$$Var(\widehat{\alpha}_j) = \frac{Var((\widehat{\beta}_y^B)_j)}{(\widehat{\beta}_x^A)_j^2} = \frac{(1-\alpha^2-\gamma_{y_j}^2)}{n_B \cdot (\widehat{\beta}_x^A)_j^2} \tag{4}$$

Hence the weights (w_j) of IV j for estimating the IVW causal effect are:

$$w_j = \frac{1}{Var(\widehat{\alpha}_j)} = \frac{n_B \cdot (\widehat{\beta}_x^A)_j^2}{(1-\alpha^2-\gamma_{y_j}^2)} \tag{5}$$

Finally, the estimate can be written in the following form:

$$\begin{aligned}\widehat{\alpha}_{IVW} &= \frac{\sum_{j=1}^m \widehat{\alpha}_j \cdot w_j}{\sum_{j=1}^m w_j} = \frac{\sum_{j=1}^m \frac{(\widehat{\beta}_y^B)_j}{(\widehat{\beta}_x^A)_j} \times \left(\frac{(\widehat{\beta}_x^A)_j^2}{(1-\alpha^2-\gamma_{y_j}^2)}\right)}{\sum_{j=1}^m \frac{(\widehat{\beta}_x^A)_j^2}{(1-\alpha^2-\gamma_{y_j}^2)}} = \frac{\sum_{j=1}^m \frac{(\widehat{\beta}_y^B)_j \cdot (\widehat{\beta}_x^A)_j}{(1-\alpha^2-\gamma_{y_j}^2)}}{\sum_{j=1}^m \frac{(\widehat{\beta}_x^A)_j^2}{(1-\alpha^2-\gamma_{y_j}^2)}} \\ &\approx \frac{\sum_{j=1}^m \frac{(\widehat{\beta}_y^B)_j \cdot (\widehat{\beta}_x^A)_j}{(1-\alpha^2)}}{\sum_{j=1}^m \frac{(\widehat{\beta}_x^A)_j^2}{(1-\alpha^2)}} = \frac{\sum_{j=1}^m (\widehat{\beta}_y^B)_j \cdot (\widehat{\beta}_x^A)_j}{\sum_{j=1}^m (\widehat{\beta}_x^A)_j^2}\end{aligned} \tag{6}$$

Here, the last approximation is based on the realistic assumption that the individual pleiotropic effect of each SNP is very small.

To account for winner's Curse, we need to consider the probability of being selected for each genetic variant. Let us consider a threshold T ($T = -\Phi^{-1}(p/2) \approx 5.45$ for $p = 5 \times 10^{-8}$, genome-wide significance threshold, for example) and use only IVs with $|(\widehat{\beta}_x^A)_j| \cdot \sqrt{n_A} > T$. By denoting $S_j := \left\{|(\widehat{\beta}_x^A)_j| \cdot \sqrt{n_A} > T\right\}$, the causal effect estimate (Equation 6) changes to:

$$\widehat{\alpha}_{IVW} \approx \frac{\sum_{j=1}^M ((\widehat{\beta}_y^B)_j \,|S_j) \cdot ((\widehat{\beta}_x^A)_j \,|S_j) \cdot Pr(S_j)}{\sum_{j=1}^M ((\widehat{\beta}_x^A)_j \,|S_j)^2 \cdot Pr(S_j)} \tag{7}$$

Note that while m denoted the number of IVs, M represents the number of genome-wide variants from which IVs are selected. By approximating the expectation of a ratio by the ratio of expectations, the expectation of the causal effect estimate (Equation 7) can be written as:

$$\begin{aligned}E[\widehat{\alpha}_{IVW}] &\approx \frac{\sum_{j=1}^M E\left[((\widehat{\beta}_y^B)_j \,|S_j) \cdot ((\widehat{\beta}_x^A)_j \,|S_j)\right] \cdot Pr(S_j)}{\sum_{j=1}^M E\left[((\widehat{\beta}_x^A)_j \,|S_j)^2\right] \cdot Pr(S_j)} \\ &= \frac{\sum_{j=1}^M s_j \cdot Pr(S_j)}{\sum_{j=1}^M t_j \cdot Pr(S_j)}\end{aligned} \tag{8}$$

The values of s_j, t_j and $Pr(S_j)$ can be analytically derived (presented in Supplementary Section

A), and we show that Equation 8 translates to:

$$E[\hat{\alpha}_{IVW}] \approx \alpha \cdot \frac{\left(\pi_x \cdot \sigma_x^2\right) \cdot \left(2 \cdot a + b \cdot (1 + n_A \cdot \sigma_x^2)\right)}{d(n_A, T, \pi_x, \sigma_x^2)} + \lambda' \cdot \frac{\pi_x \cdot \left(2 \cdot a + b \cdot (1 + n_A \cdot \sigma_x^2)\right) + \left(1 - \pi_x\right) \cdot 2 \cdot c}{d(n_A, T, \pi_x, \sigma_x^2)} \tag{9}$$

where π_x and σ_x^2 are characteristics of the genetic architecture of trait X (respectively, a measure of the polygenicity and the per-variant heritability, see Equation S35)[16] and λ' is a quantity closely related to the cross-trait LD score regression (LDSC) intercept[17] (λ) : $\lambda' = \frac{\lambda}{\sqrt{n_A \cdot n_B}}$. The constants a, b and c do not depend on the causal effect α nor the sample overlap since $n_{A \cap B}$ is only affecting λ' (see Equations S33 and S51). The same is true for the denominator ($d(n_A, T, \pi_x, \sigma_x^2)$) (see Equation S52).

We can see that in the absence of sample overlap, the second term is equal to 0, and the first term corresponds to the causal effect estimate. In this case, $E[\hat{\alpha}_{IVW}]$ is lower than α and the IVW-based effect will be biased towards the null (Figure S1). The only parameters affecting the bias are π_x and h_x^2, n_A and T. When π_x is smaller, or h_x^2 is larger, then IVs have stronger effects, leading to a smaller bias when all other parameters are kept constant. As expected, since these are commonly used approaches to limit weak instrument bias, using a more stringent threshold and increasing the exposure sample size both reduce the bias.
When there is sample overlap (with % overlap defined as $\frac{n_{A \cap B}}{\sqrt{n_A \cdot n_B}}$), the expression of $E[\hat{\alpha}_{IVW}]$ is more complex. The magnitude of the bias will not only depend on the parameters described for non-overlapping samples (Figures S2, S3 and S4), but also on the confounder's effect (ρ) that can affect both the magnitude and the direction of the bias. For example, when the percentage of overlap is relatively low (20%) and ρ has the same sign as α, then the estimate will be further biased towards the null, whereas a confounder acting in the opposite direction (as the causal effect) will reduce the bias (Figure S2). When the percentage of overlap increases (Figures S3 and S4), the effect of sample overlap gets stronger, and the bias direction strongly depends on ρ.

All parameters except α are known or can be estimated from the data. We assume that sample sizes for X and Y (respectively n_A and n_B) are known, as well as the threshold used to select IVs (T). Parameter λ' can be estimated from cross-trait LDSC[17]. π_x and σ_x^2 are estimated by matching the denominator of this formula, $d(n_A, T, \pi_x, \sigma_x^2)$, to the denominator of equation 8 (see Supplementary Section B for details).
From Equation 10, we can derive a corrected effect for the causal effect :

$$\hat{\alpha}_c = \frac{\hat{\alpha}_{IVW} \cdot d(n_A, T, \pi_x, \sigma_x^2) - \lambda' \cdot (\pi_x \cdot \left(2 \cdot a + b \cdot (1 + \sigma_x^2 \cdot n_A)\right) + \left(1 - \pi_x\right) \cdot 2 \cdot c)}{\left(\pi_x \cdot \sigma_x^2\right) \cdot \left(2 \cdot a + b \cdot (1 + n_A \cdot \sigma_x^2)\right)} \tag{10}$$

We also derived the standard error of the corrected effect as well as the covariance between IVW-based and corrected effects in Supplementary Section C. Note that the formula proposed

by Burgess *et al.*[4] (Supplementary Section D) under the null is a special case of ours when all instruments are selected based on external data (i.e. there is no winner's curse).
This approach has been implemented in an R-package (`MRlap`), using existing functions from the Two-Sample MR R-package[18] and the LDSC implementation from the GenomicSEM R-package[19]. All analyses presented in this paper have been performed using version 0.0.2. IVs were selected with a varying T threshold and distance-pruned (500kb). For the LDSC analyses we used the 1000G LD-scores[20].

2.2 Simulations

We used UKBB[15] genotypic data and restricted our analyses to unrelated individuals of British ancestry (identified using genomic principal components) and HapMap3 genetic variants[21] ($M \approx 1,150,000$) to simulate phenotypic data. From this set of 379,530 individuals, we first sampled the exposure dataset (n_A individuals) and 5 different outcome datasets of sample size n_B, with an overlap with the exposure dataset varying (from no overlap to full overlap, increasing in increments of 25%). Next, causal SNPs for the exposure were randomly drawn from the set of 1,150,000 genetic variants, based on the polygenicity of X (π_x) and their effects were simulated using the heritability of X (h_x^2) as follows:

$$\boldsymbol{\gamma_x} \sim \begin{cases} 0 & \text{with probability } 1-\pi_x\text{, for non-causal variants} \\ \mathcal{N}\left(0, \frac{h_x^2}{M \cdot \pi_x}\right) & \text{with probability } \pi_x\text{, for causal variants} \end{cases} \tag{11}$$

For simplicity, we assumed that there were no direct genetic effects on the outcome. Then, phenotypic data for X and Y were simulated for all individuals included in the exposure or in any of the outcome samples, taking into account the effect of the confounder U on X and Y (respectively κ_x and κ_y) and the causal effect of X on Y (α), using the following design:

$$\boldsymbol{u} \sim \mathcal{N}(0,1) \tag{12}$$

$$\boldsymbol{x} = G \cdot \boldsymbol{\gamma_x} + \boldsymbol{u} \cdot \kappa_x + \epsilon_x \tag{13}$$

$$\text{with } \epsilon_x \sim \mathcal{N}(0, 1-(h_x^2+\kappa_x^2))$$

$$\boldsymbol{y} = \alpha \cdot \boldsymbol{x} + \boldsymbol{u} \cdot \kappa_y + \epsilon_y \tag{14}$$

$$\text{with } \epsilon_y \sim \mathcal{N}(0, 1-(\alpha^2+\kappa_y^2+2\cdot\alpha\cdot\kappa_y\cdot\kappa_x))$$

Note that this design ensures that both X and Y have a zero mean and a variance of 1. Slightly different designs, with the same property, were used to simulate phenotypic data when the exposure was binary (case-control) and in the presence of uncorrelated or correlated pleiotropy (Supplementary Section E). A GWAS was performed for each sample (one for the exposure and five GWASs for the outcome, one for each sample overlap) using BGENIE[22]. We then applied MRlap to these GWAS summary statistics to obtain the IVW-based and the corrected effect estimates.

For each parameter setting we tested, 100 datasets were simulated. Our standard parameter

settings consisted of simulating data for $n_A = 20,000$ and $n_B = 20,000$ individuals. X was simulated with moderate polygenicity and large heritability ($\pi_x = 0.001$ and $h_x^2 = 0.4$). U had a moderate effect on both X and Y ($\kappa_x = 0.3$, $\kappa_y = 0.5$), leading to a correlation (ρ) of 0.15 induced by the confounder. We varied the size of the causal effect of X on Y from null ($\alpha = 0$) to moderate ($\alpha = 0.2$).

In addition to these standard settings, we explored various other parameter values. We investigated the effect of a confounder acting in an opposite direction ($\kappa_x = -0.3$ and $\kappa_y = 0.5$) and tested different strengths for the confounding factor (weaker : $\kappa_x = 0.15$ and $\kappa_y = 0.3$, and stronger : $\kappa_x = 0.5$ and $\kappa_y = 0.8$). We also considered a case-control design, where the exposure was first simulated on the liability scale before being converted to the observed scale for our analyses. For this setting we used larger sample sizes ($n_A = 100,000$, $n_B = 100,000$) and a prevalence of 0.1 to define cases and controls. We explored a scenario with more realistic parameters: larger sample sizes ($n_A = 100,000$, $n_B = 100,000$), increased polygenicity ($\pi_x = 0.005$), lower heritability ($h_x^2 = 0.2$) and a smaller causal effect ($\alpha = 0.1$). Finally, we simulated data in presence of both uncorrelated and correlated pleiotropy. For the uncorrelated pleiotropy, we used the standard settings parameters and added direct genetic effects on Y ($\pi_y = 0.002$ and $h_y^2 = 0.3$). 60% of the SNPs having a direct effect on X were also directly affecting Y and their direct effects on each trait were uncorrelated. To simulate data in presence of correlated pleiotropy, we added to the model a genetic confounder (U_g) acting both on X and Y, with respective effects q_x and q_y. First, we modelled a genetic confounder that was highly polygenic and fairly heritable ($\pi_u = 0.0001$ and $h_u^2 = 0.2$), with moderate effects on the two traits ($q_x = 0.4$ and $q_y = 0.3$). Then we slightly increased the confounder's polygenicity and, in order to make the genetic confounding effect stronger, we increased its heritability and its effects on X and Y ($\pi_u = 0.0005$, $h_u^2 = 0.3$, $q_x = 0.5$ and $q_y = 0.7$).

For each scenario, IVW-based and corrected causal effects were compared for different degrees of sample overlap and different instrument selection thresholds. Results quality was assessed using root-mean square error (RMSE).

For a given instrument selection threshold, we obtained 500 causal effect estimates: one for each of the 100 simulated data sets for each of the five degrees of sample overlap (ranging from 0 to 100%). Causal effect estimates should ideally not depend on the extent of overlap between the exposure and outcome samples. To quantify the extent to which this holds, we grouped estimates according to which sample overlap degree they came from and compared the between group variance relative to the within group variance of the estimates. A method that is robust to overlap between the exposure and outcome samples will have small between group variance relative to the variance of the estimator (characterised by the within group variance).

Finally, we tested for differences between IVW-based and corrected effects using the following test statistic:

$$t_{\text{diff}} = \frac{\hat{\alpha}_{IVW} - \hat{\alpha}_c}{\sqrt{Var(\hat{\alpha}_{IVW}) + Var(\hat{\alpha}_c) - 2 \cdot Cov(\hat{\alpha}_{IVW}, \hat{\alpha}_c)}} \tag{15}$$

In addition to those scenarios, we wanted to assess the potential gain, in terms of bias and variance, arising from the possibility of performing analyses using the full UKBB sample instead of having to split it into two halves to avoid sample overlap. To do so, we simulated data using the following parameters : large polygenicity and moderate heritability ($\pi_x = 0.01$ and $h_x^2 = 0.15$), moderate confounder effect ($\kappa_x = 0.3$, $\kappa_y = 0.5$), and a fairly small causal effect ($\alpha = 0.1$). In this case, we only compared the IVW-based causal effect estimates from non-overlapping samples ($n_A = 180,000$, $n_B = 180,000$, roughly half of UKBB sample size) and the corrected causal effect estimates from fully overlapping samples ($n_A = 360,000$, $n_B = 360,000$) by measuring the bias, the variance and the RMSE.

Finally, we also compared our `MRlap` results to those of pleiotropy-robust approaches, such as weighted median[13], weighted mode[14] (for two thresholds, $5e-8$ and $1e-6$) and MR-RAPS[23] (for the default threshold of $1e-4$), both under the standard settings and in all scenarios with pleiotropy, for all degrees of sample overlap.

2.3 Application to UKBB

2.3.1 Analyses using a sampling strategy

To assess the effect of sample overlap on real data, we used a design very similar to the one used for simulations. We used both genotypic and phenotypic data from UKBB[15] and restricted our analyses to the same subsets of individuals and genetic variants. From this set of individuals, we first sampled the exposure dataset (100,000 individuals) and 5 different outcome datasets (100,000 individuals), where the overlap with the exposure dataset varied (from no overlap to full overlap, increasing in increments of 25% - in the case of unequal sample sizes, the percentage of overlap for the samples will proportionally increase from 0 to the maximum value attainable given the difference in sample sizes). Note that we always used the full set of individuals to create the 100,000-individuals samples, so the percentage of missing data in each sample will be the same as in the UKBB. Therefore, the total number of individuals with phenotypic data (effective sample size) will vary depending on the traits. We assessed the effect of body mass index (BMI) on systolic blood pressure (SBP), on the number of cigarettes previously smoked daily (smoking) and on alcohol intake frequency (alcohol). For smoking, answers "prefer not answer" and "less than one a day" were considered as missing. For alcohol, answers were recoded to correspond to an increased intake frequency, and answers "do not know" were considered missing. Details about the pairs of traits analysed are available in Table S1.
Phenotypic data was normalised (inverse-normal quantile transformed) and subsequently adjusted for the following covariates: sex, age, age×age and the first 40 principal components. Similarly to what we did for simulations, a GWAS was performed for each sample (the exposure dataset and the five outcome datasets, one for each sample overlap) using BGENIE[22]. We then applied `MRlap` to these GWAS summary statistics to obtain the IVW-based and the corrected effect estimates. In addition, since in this case a reverse causal effect (from the outcome on the exposure) could exist, we filtered out variants that were significantly more strongly associated

with the outcome than with the exposure in order to remove potentially invalid IVs (Steiger filter).

We repeated this sampling approach a 100 times. For each repetition, IVW-based and corrected causal effects were compared using the within groups and between groups variances and we tested for differences between IVW-based and corrected effects using Equation 15.

2.3.2 Analyses using the full UKBB sample

We used GWAS summary statistics made available by the Neale Lab[24] for 17 traits (Table S2) and analysed all pairwise relationships (262 analyses in total). We used `MRlap` to obtain the IVW-based and the corrected effect estimates. In this case, IVs were selected using a p-value threshold of $5e-8$. In addition, since in this case a reverse causal effect (from the outcome on the exposure) could exist, we filtered out variants that were significantly more strongly associated with the outcome than with the exposure in order to remove potentially invalid IVs (Steiger filter).

For each pair, the significance of the difference between IVW-based and causal effect was assessed using a Bonferroni adjusted threshold to account for multiple testing (p-value $\leq 0.05/262$). We also reported the strength of the bias towards the null induced by the first term of Equation 10. In addition, we used the UKBB phenotypic data to estimate the observational correlation for all trait pairs. Observational correlation estimates could then be compared to (bidirectional) standardised causal effect estimates and help with the interpretation of the confounding effect.

3 Results

3.1 Overview of the method

We propose a two-sample MR framework that takes into account two sources of bias : weak instrument bias and winner's curse, while simultaneously accounting for potential sample overlap and its effect as a modifier of these biases. We analytically derived the expectation of the observed effect for IVW-based MR estimate:

$$E[\widehat{\alpha}_{IVW}] = f(\alpha, n_A, n_B, T, \pi_X, \sigma_X^2, \lambda)$$

which depends on the true causal effect size (α), the sample sizes of the exposure and outcome GWASs (n_A, n_B), the threshold used to select IVs (T), the cross-trait LDSC intercept (λ, which depends on the degree of the sample overlap, the true causal effect and the strength of the confounder) and the genetic architecture of the exposure (polygenicity π_X and per-variant heritability $\sigma_X^2 = \frac{h_X^2}{M \cdot \pi_X}$, M being the number of potential IVs). All parameters (except α) are either known or can be estimated from the data. This allows us to adjust the IVW causal effect estimate with the aim of making it unbiased.

3.2 Simulations

Simulation results under our standard settings show a large discrepancy of the IVW-based causal effects estimated using different degrees of sample overlap, while the corrected effects are more closely aligned with the true causal effects (Figure 2 - A, Table S3). We observe a 10% overestimation of the causal effect for fully overlapping samples, and a 15% underestimation of the causal effect for non-overlapping samples, due to winner's curse and weak instrument bias both biasing the estimate towards the null. As expected from Equation 10, the bias is larger when using less stringent thresholds T. For all the thresholds tested, the ratio of the between groups and the within groups variances is larger (up to 26 times) for IVW-based effects than for corrected effects (Table S4), highlighting the differences in IVW-based effects when estimated using different degrees of overlaps. The fact that the within group variance is 1.3 times higher for the corrected effects is due to the slightly increased variance of the bias-corrected estimator. The RMSE of the IVW-based effects is very dependent on the degree of overlap (being larger for non-overlapping and fully overlapping samples) while the RMSE of the corrected effects is consistent across varying degrees of overlap and up to 1.75 times lower for non-overlapping samples (Figure 2 - B). The corrected effects are significantly different from the IVW-based effects for all overlaps values except 50% and all thresholds (Table S3). The absolute bias of the IVW-based effects goes up to 0.033 while for the corrected effect it is smaller than 0.012 for all overlaps and thresholds.

The bias of IVW-based effects depends on the strength of the confounder. If the confounder is weak, IVW-based effects are mostly biased towards the null for low overlaps (Figure S5, Table S5, Table S6). When we simulate a stronger confounder, the bias of the IVW-based effects for

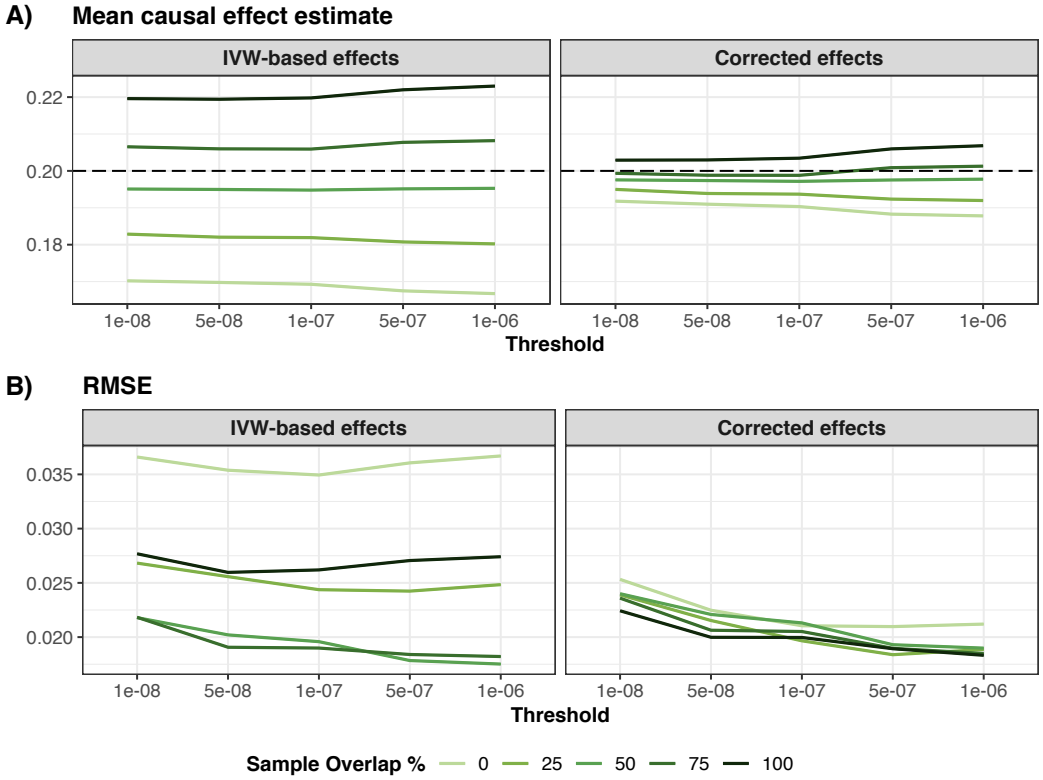

Figure 2: Simulation results for standard settings
$n_A = n_B = 20,000, \pi_x = 0.001, h_x^2 = 0.4, \kappa_x = 0.3, \kappa_y = 0.5, \alpha = 0.2$
Panel A) shows the mean IVW-based and corrected effect for each overlap and threshold obtained from 100 simulations (the dashed line represents the true causal effect). Panel B) shows the mean RMSE obtained for IVW-based and corrected effect for each overlap and threshold.

fully overlapping samples increases (Figure S6, Table S7, Table S8). In both cases, the corrected and the IVW-based effects are significantly different for almost all overlaps and thresholds, and the corrected effects are substantially less biased than the IVW-based effects (Table S5, Table S7).

When the confounder effect (ρ) and the causal effect (α) are acting in opposite directions, the results are particularly interesting because winner's curse and weak instrument bias are biasing the results towards the null regardless of the sample overlap degree (Figure 3 - A, Table S9). In this case, IVW-based effects are more similar across the different degrees of sample overlap tested, but all are underestimating the true causal effect. For this reason, we do not observe such a large decrease in the heterogeneity of the estimates across different sample overlaps compared to the corrected effects (ratio between the between groups and the within groups variance is 5 times larger for IVW-based effects), but the latter ones show lower RMSE and bias for all overlaps and thresholds (Figure 3 - B, Table S10). In this scenario, IVW-based and corrected effects significantly differ for all overlaps and thresholds, with an average underestimation of 13% for IVW-based effects (Table S9).

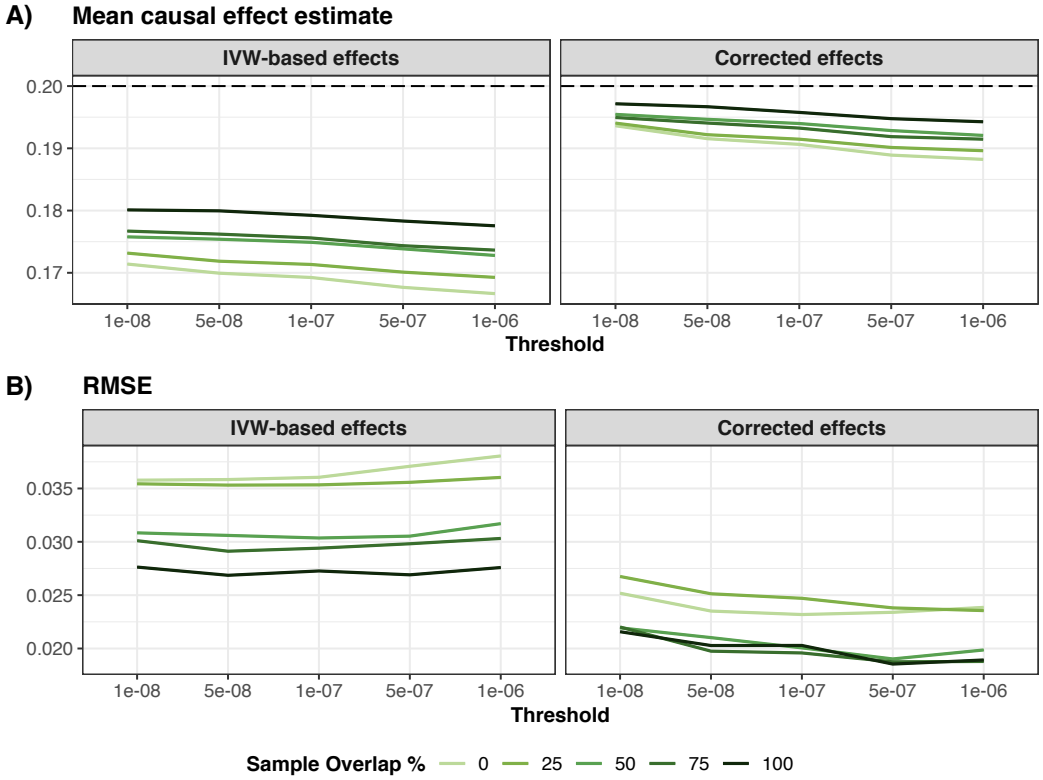

Figure 3: Simulation results for a scenario with a negative confounder
$n_A = n_B = 20,000, \pi_x = 0.001, h_x^2 = 0.4, \kappa_x = -0.3, \kappa_y = 0.5, \alpha = 0.2$
Panel A) shows the mean IVW-based and corrected effect for each overlap and threshold obtained from 100 simulations (the dashed line represents the true causal effect). Panel B) shows the mean RMSE obtained for IVW-based and corrected effect for each overlap and threshold.

In the absence of a true causal relationship between the exposure and the outcome, IVW-based effects from non-overlapping samples are unbiased. However, for fully overlapping samples, the IVW-based effects are biased towards the confounder's effect (Figure S7, Table S11, Table S12). We showed that for large overlap percentages ($\geq 50\%$), the corrected effects are significantly different from the IVW-based effects (60% smaller), and they are less biased for all overlaps and thresholds. Moreover, while the false positive rate for IVW-based effects using non-overlapping samples is below 5%, it is much larger (between 20 and 40% depending on the threshold) when using fully overlapping samples. The correction proposed here provides a much better control of false positive rate for all degrees of sample overlap (Figure S8).

Simulating binary, instead of continuous, exposure led to very similar results. The absolute bias was in average 2.3 times larger for IVW-based effects than for corrected effects (Table S13) and the causal effect estimates were much more consistent across varying degrees of overlap after correction (Figure S9, Table S14). Results obtained using more realistic parameters in terms of sample sizes, genetic architecture and causal effect strength show a similar pattern. We observe an important bias of IVW-based causal effects, mostly when estimated from non-overlapping (22% underestimation) or fully overlapping samples (30% overestimation), that is

strongly reduced when using our correction (Figure S10, Table S15, Table S16). Corrected effects significantly differ from IVW-based effects for the most extreme overlaps values (0%, 75%, 100%) for which corrected effects are on average 5 times less biased than IVW-based effects.

We compared corrected effects obtained using the full sample to IVW-based effects obtained by splitting it into two halves to avoid sample overlap. We showed that the IVW estimators were 3.27 times more biased than those from `MRlap` and the latter reduced the estimator variance by more than three-fold (3.43). Thus we have demonstrated that applying `MRlap` to the full (overlapping) sample is a better strategy than ensuring no sample overlap and applying the IVW estimator, because it is less biased and has considerably lower variance (Table S17).

We also investigated the extent of the bias, for both IVW-based and corrected effects, in scenarios with either uncorrelated pleiotropy (Table S18, Figure S11, Table S19), moderate correlated pleiotropy (Table S20, Figure S12, Table S21) and strong correlated pleiotropy (Table S22, Figure S13, Table S23). Results obtained in presence of uncorrelated pleiotropy are very similar to the ones obtained for the standard settings, with slightly larger within group variances. In presence of correlated pleiotropy however, we observe a bias towards the ratio of the genetic confounder effects on Y and X (0.75 for moderate correlated pleiotropy and 1.4 for strong correlated pleiotropy) for both IVW-based and corrected effects for all sample overlap degrees. This can be explained by the fact that there is now a third source of bias, correlated pleiotropy, affecting the causal effect estimates. This source of bias is independent of sample overlap, and corrected effect estimates are able to recover consistent causal effect estimates across varying degrees of overlap, corresponding to the sum of the true causal effect and of the bias induced by correlated pleiotropy. When comparing our results to results obtained using pleiotropy-robust methods (Figure 4, Figure S14), we observe that for non-overlapping samples, Weighted Median, Weighted Mode and MR-RAPS estimates are able to recover the value of the IVW-based estimate from the standard settings scenarios (i.e. a downward biased causal effect estimate). However, for large degrees of sample overlap ($\geq$ 50%), these approaches are more biased than IVW and `MRlap`.

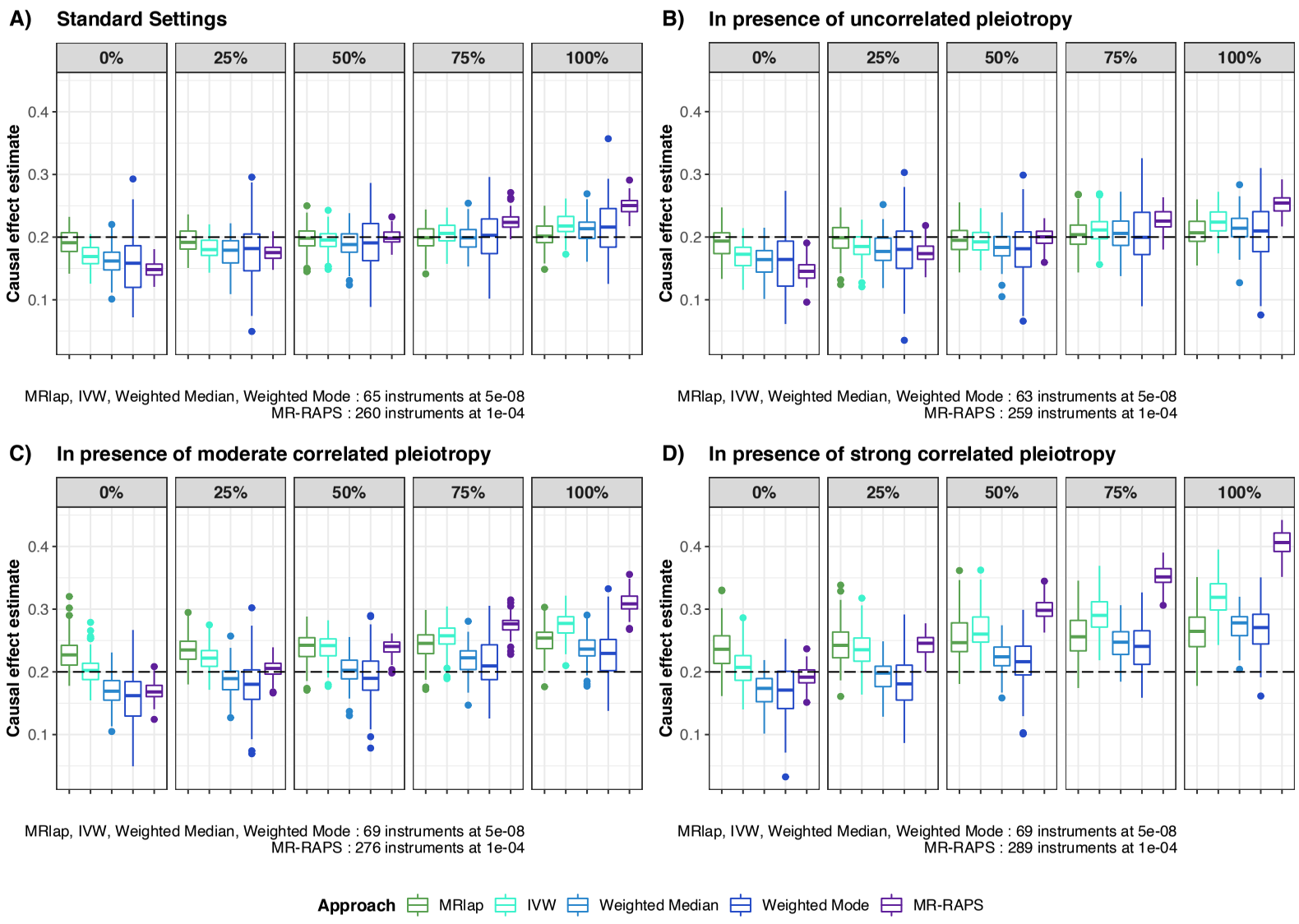

Figure 4: Comparison of different MR approaches
Causal effects estimates were obtained from 100 simulations using 5 different approaches (MRlap in green, IVW in turquoise blue, Weighted Median in light blue, Weighted Mode in dark blue and MR-RAPS in purple). The dashed line represents the true causal effect. Panel A) shows results for the standard settings scenario. Panel B) shows results in presence of uncorrelated pleiotropy. Panel C) shows results in presence of moderate correlated pleiotropy. Panel D) shows results in presence of strong correlated pleiotropy. At the bottom of each panel, we indicated the average number of instruments (at 5e-08 / 1e-04) for the different approaches.

3.3 Application to UKBB

We tested our method on UKBB obesity-related traits using a similar approach and splitting the full dataset into samples of varying degrees of overlap. When looking at the IVW-based effect of BMI on SBP (Figure 5 - A), we observed that the estimates obtained using different p-value thresholds vary considerably, independently of sample overlap. Even though we expect an increase in bias when reducing the threshold used, as shown in simulations, here we see that for non-overlapping sample the IVW-based effect is larger for less stringent thresholds. This is inconsistent with winner's curse and weak instrument bias that lean the estimate towards the null and we would expect the IVW-based effects for less stringent thresholds to be smaller (in absolute value). Hence, we believe that this phenomenon is not related to any of the biases discussed here and is due to other reasons such as the existence of multiple causal effects depending on exposure sub-type or the presence of a heritable confounder (see Discussion). Here we will focus on the results obtained using a p-value threshold of 5e-8.

When using IVs reaching genome-wide significance, the IVW-based effects range between 0.095, for non-overlapping samples, and 0.129, for fully overlapping samples (Table S24). After correction, the range of the estimated effect is about 2 times smaller (0.108 - 0.128). The better agreement of corrected effects across overlaps can be seen by looking at the ratio between the between groups and the within groups variance which is 4 times larger for IVW-based effects (Table S25). For non-overlapping samples, the difference between IVW-based and corrected effect is significant ($p_{\text{diff}} = 0.0049$), and standard two-sample MR underestimates the causal effect by about 25% (the IVW-based effect is 0.095 while the corrected effect is 0.126). For fully overlapping samples, there is no significant difference between IVW-based and corrected effects. We observed a similar pattern when investigating the effect of BMI on smoking (Figure S15 - A, Table S26 , Table S27) where the largest bias occurs for non-overlapping samples. The IVW-based causal effect at p=5e-8 is 0.130 for non-overlapping samples while results after correction point towards a causal effect of 0.170 (underestimation of 24% - significant difference between IVW-based and corrected effects, $p_{\text{diff}} = 0.0137$). We do not see a significant difference between IVW-based and corrected effects for larger overlap values, but it is important to note that in this case, because of the impact of missing data on our design, the largest possible overlap was only 48.5%.

Our results implicate the existence of an environmental confounder biasing the causal effect estimate of BMI on alcohol intake frequency. At p=5e-8, IVW-based effects range between -0.187 for non-overlapping samples and -0.218 for fully overlapping samples, whereas the corrected effects are larger (-0.244 to -0.2515) (Figure 5 - B, Table S28). The between groups variance is much smaller for corrected effects (Table S29). The difference between IVW-based and corrected effects is significant for all overlaps and the corrected effects being stronger than the IVW-based ones hints at the existence of an environmental confounder having a concordant effect on BMI and alcohol intake frequency, biasing all estimates towards the null (as shown in simulations, Figure 3). While we could not identify any plausible confounder of this relationship, its exis-

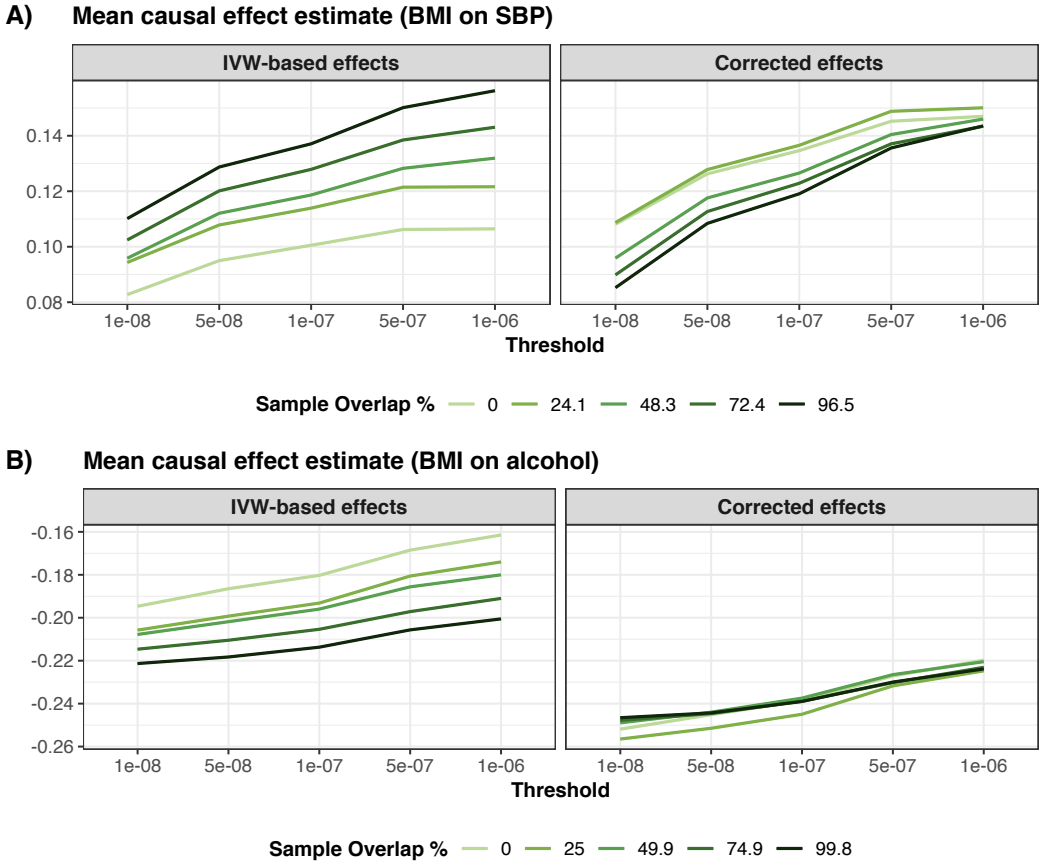

Figure 5: Effect of BMI on SBP and effect of BMI on alcohol intake frequency
This figure shows the mean IVW-based and corrected effect for each overlap and threshold obtained from 100 different sampled datasets. Panel A) corresponds to the effect estimates of BMI on SBP. Panel B) corresponds to the effect estimates of BMI on alcohol.

tence is supported by the fact that the observational correlation between BMI and alcohol intake frequency (-0.13 among the 379,530 genetically British individuals in the UKBB) is weaker than the standardised causal effect.

We also applied our method to estimate all pairwise bidirectional causal effects between 17 traits based on summary statistics from the UKBB. For 60 out the 262 relationships, the difference between the IVW-based and the corrected effect was significant (p-value $< 0.05/262$, Table S30). For 19 of these 60 directional pairs, neither the IVW-based, nor the corrected effects were significant ($< 0.05/262$) and hence we focused our analysis on the 41 remaining relationships. There is overall a good agreement between IVW-based and corrected effects (Figure 6 - A). For 18 relationships, the relative difference between the IVW-based and the corrected effect was larger than 15%. Surprisingly, for a large proportion of relationships (21/41), the corrected effect was stronger than the IVW-based effect, despite the large degree of sample overlap. This can be explained by the existence of a confounder (environmental or genetic) acting in the opposite direction as the causal effect and for all these relationships. This conclusion from our model-fitting was supported by the fact that the observational correlation between the traits was

weaker than the standardised causal effect estimates (Table S31). In these situations the large sample overlap drives the IVW estimates towards the (weaker) observational correlation. This is for example the case of the negative causal effect of SBP on LDL cholesterol that appears to be stronger after correction ($\hat{\alpha}_{IVW} = -0.1109, \hat{\alpha}_c = -0.1468$). Based on our own MR results, we identified two potential confounders : triglycerides levels and (potentially parental) height. These two traits have a significant positive causal effect on both SBP and LDL cholesterol that could lead to a positive confounding effect. For 20 relationships, IVW-based effects were biased upwards, which is consistent with the existence of a confounder acting in the same direction, and leading to a stronger observational correlation. BMI, for example, could be responsible of the overestimation of the effect of triglycerides on SBP ($\hat{\alpha}_{IVW} = 0.0576, \hat{\alpha}_c = 0.0517$) and on C-reactive proteins levels ($\hat{\alpha}_{IVW} = 0.1204, \hat{\alpha}_c = 0.1101$).

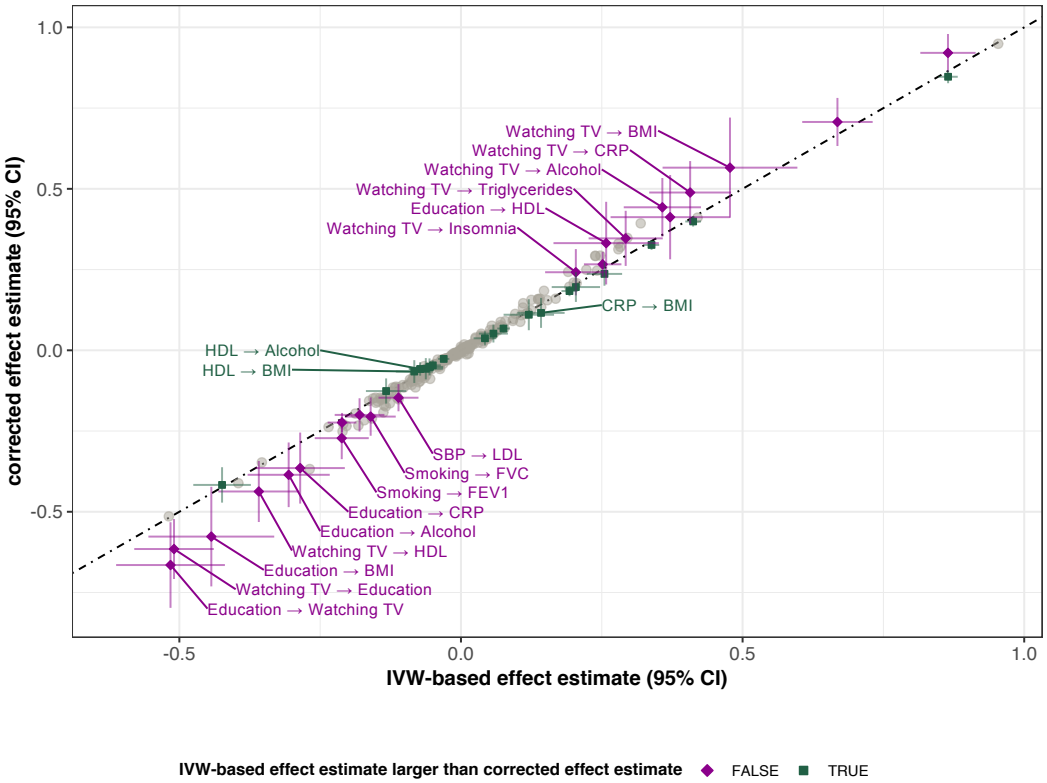

Figure 6: Comparison of IVW-based and corrected effects for 262 relationships
The corrected causal effect estimates are compared to the IVW-based causal effect estimates for each relationship. Causal effects that do not significantly differ before and after correction, as well as relationships for which both IVW-based and corrected effects are insignificant are shown in grey. Causal effect that are stronger before correction (upward bias) are in green (squares) and causal effects that are weaker before correction (downward bias) are in purple (diamonds). Relationships for which the relative difference between the IVW-based and the corrected effect is larger than 15% are labelled.

4 Discussion

We developed a method that models the entire instrument selection process, sample overlap and exposure effect estimation error. As a result, our approach reduces winner's curse and weak instrument bias, even when the degree of overlap is unknown. To the best of our knowledge, while some empirical simulation work describing the interplay between these biases has been done[25], no other method can tackle all the aforementioned biases while using summary statistics from exposure and outcome GWASs with arbitrary sample overlap. We tested our approach using a wide range of simulations scenarios: varying the strength of the causal effect, the strength of the confounder effect, sample sizes for the exposure and the outcome, as well as the genetic architecture of the exposure and demonstrated that both estimates for non-overlapping and fully overlapping samples can be biased. The direction and the magnitude of the bias depends on sample overlap and is strongly influenced by the effect of the confounder. When the confounder and the causal effect are acting in the same direction, observed effects are overestimated for fully-overlapping samples and underestimated for non-overlapping samples. However, when they are acting in opposite directions, these are underestimated for all overlaps because the direction of the biases is towards the null for any degree of sample overlap. We also showed that in the absence of a causal effect, results from overlapping samples would be biased, potentially leading to elevated type I error.

The correction we proposed worked remarkably well under all scenarios, and allows to drastically reduce the bias. For standard settings for example, we observed a 15% overestimation for fully overlapping samples and 10% underestimation for non-overlapping samples, that were respectively reduced to a 5% overestimation and a 2% underestimation after correction. We also found significant differences between IVW-based and corrected effects for fully-overlapping samples under all scenarios. For non-overlapping samples, IVW-based and corrected effects were significantly different under all scenarios expect in the case of the absence of causal effect. The decreased bias comes with increased variance (due to the correction) however in all of our simulation scenarios it reduced estimation error (RMSE) for at least one, if not all, sample overlap degrees. Moreover, while the RMSE of IVW-based effects strongly depends on the degree of overlap (because the bias is overlap-dependent), the RMSE of corrected effects is very similar for all overlaps.

For real data, we first used a sampling strategy to compare results obtained using varying degrees of sample overlap. Strong discrepancies were observed for low degrees overlap in the three relationships we looked at, with significant differences between IVW-based and corrected effects. This means that standard two-sample MR settings often lead to an underestimation of the true causal effect, that can be corrected using our approach. We also demonstrated that while most studies are extremely keen on avoiding any sample overlap while performing two-sample MR analysis fearing potential bias, the bias is often much less substantial for higher degrees of sample overlap. Among our many examples, we found that the IVW-MR estimate for the effect of BMI on alcohol intake frequency using the fully-overlapping samples is biased by a confounder.

In this case, the confounder and the causal effect were acting in opposite direction, leading to an underestimation of the IVW-based effect for all overlaps. We have also highlighted that there is an important heterogeneity in causal effect estimates that vary with the IV selection threshold, due to heterogeneity in the estimates between the groups of genetic variants used for different thresholds. This can happen if there is a strong phenotypic heterogeneity in the exposure, in which case different groups of IVs could be affecting the exposure through different pathways[26]. Alternatively, in the presence of a genetic confounder, IVs picked up at a less stringent thresholds may be associated to the confounder, hence violating the second assumption of MR. Such phenomenon is out of the scope of our paper. In such case, IVW two-sample MR estimates would be biased, and more sophisticated approaches either specifically accounting for this genetic confounding (CAUSE[27], LHC-MR[28]) or others allowing for multiple causal effects (MR-Clust[26]) would be needed.

We also used our approach to estimate all pairwise bidirectional causal effects between 17 traits based on summary statistics from the UKBB. For a large majority of the traits (221/261), we did not detect any significant difference between IVW-based and corrected effects. This is consistent with the fact that the effect of the biases will decrease as the sample size increases, and for very large sample sizes, such as UKBB, this bias is expected to be less pronounced. However, we showed that the overall bias is also dependent on the genetic architecture of the exposure, and low heritability and/or highly polygenic traits will be more strongly affected. Indeed, amongst the relationships with significant differences, the exposure traits were often the ones with the largest polygenicity (7 relationships with height as exposure, 2 relationships with BMI as exposure) and the ones with the lowest heritability (7 relationships with Watching TV as exposure, 5 relationships with education as exposure). We also observed that the strength of the bias was in general stronger when a confounder acting in the opposite direction as the causal effect existed.

Our approach has its own limitations. As IVW-MR estimates, our corrected effect estimates will also be biased in case of the existence of a genetic confounder through which some of the selected instruments are primarily acting on the exposure, as shown in our simulations with correlated pleiotropy. In addition, our analytical derivation hinges on a genetic architecture of the exposure, namely assuming a spike and slab distribution of the multivariable effect sizes. Although this is a widely used and confirmed polygenic model, deviations from it could reduce the efficiency of our bias correction. It is also important to note that our work focused on continuous traits, and our approach would only work using case-control designs if the sample overlap degree do not differ between cases and controls.

Nowadays, samples from large biobanks are often used to estimate SNP effect sizes for both the exposure and the outcome, and hence it will be less and less possible to ensure that the two samples used are not overlapping. Thus, the need for non-overlapping samples forces researchers to use summary statistics from reduced sample size. Estimating the corrected effect using our approach (implemented in an R-package to facilitate its use) can be performed as a sensitivity

analysis: if the corrected effect does not significantly differ from the IVW-based effect, then the IVW-MR estimate can be safely used (with the advantage of having lower variance). However, if there is a significant difference, corrected effects should be preferred as they are be less biased, independently of the sample overlap.

Author contributions

Z.K. designed and supervised the project. Z.K. and N.M. developed the methods. N.M. performed the analyses. N.M. and Z.K wrote the manuscript.

Data availability

Most of the data underlying this article are available in the article and in its online supplementary material. We also used the UK Biobank Resource for genetic and phenotypic data.
The `MRlap` R-package is freely available under a GPL-2 License, and can be accessed, alongside with user guides and tutorials, from `https://github.com/n-mounier/MRlap`.

Supplementary data

Supplementary data are available online.

Funding

Z.K. was funded by the Swiss National Science Foundation (# 310030-189147) and the Department of Computational Biology (UNIL).

Acknowledgements

The authors thank Chiara Auwerx, Liza Darrous, Sven Erik Ojavee, Marion Patxot, Eleonora Porcu, Marie Sadler and Tabea Schoeler for the helpful discussions and relevant comments.
Computations have been performed on the HPC cluster of the Lausanne University Hospital.
This research has been conducted using the UK Biobank Resource under Application Number 16389.

Conflict of interest

None declared.

References

[1] Lawlor DA, Harbord RM, Sterne JAC, Timpson N, Smith GD. Mendelian randomization: Using genes as instruments for making causal inferences in epidemiology. *Stat Med* 2008;**27**:1133-63. Available from: https://doi.org/10.1002/sim.3034.

[2] Burgess S, Butterworth A, Thompson SG. Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data. *Genet Epidemiol* 2013;**37**:658-65. Available from: https://doi.org/10.1002/gepi.21758.

[3] Visscher PM, Wray NR, Zhang Q, et al. 10 Years of GWAS Discovery: Biology, Function, and Translation. *Am J Hum Genet* 2017;**101**:5-22. Available from: https://doi.org/10.1016/j.ajhg.2017.06.005.

[4] Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol* 2016;**40**:597-608. Available from: https://doi.org/10.1002/gepi.21998.

[5] Bowden J, Del Greco FM, Minelli C, Smith GD, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol* 2016;**45**:1961-74. Available from: https://doi.org/10.1093/ije/dyw220.

[6] Burgess S, Thompson SG. Bias in causal estimates from Mendelian randomization studies with weak instruments. *Stat Med* 2011;**30**:1312-23. Available from: https://doi.org/10.1002/sim.4197.

[7] Burgess S, Thompson SG. Avoiding bias from weak instruments in Mendelian randomization studies. *Int J Epidemiol* 2011 Mar;**40**:755-64. Available from: https://doi.org/10.1093/ije/dyr036.

[8] Zheng J, Baird D, Borges MC, et al. Recent Developments in Mendelian Randomization Studies. *Curr Epidemiol Rep* 2017;**4**:330-45. Available from: https://doi.org/10.1007/s40471-017-0128-6.

[9] Bowden J, Del Greco FM, Minelli C, Smith GD, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. *Stat Med* 2017;**36**:1783-802. Available from: https://doi.org/10.1002/sim.7221.

[10] Palmer C, Pe'er I. Statistical correction of the Winner's Curse explains replication variability in quantitative trait genome-wide association studies. *PLoS Genet* 2017;**13**:1-18. Available from: https://doi.org/10.1371/journal.pgen.1006916.

[11] Zhong H, Prentice RL. Bias-reduced estimators and confidence intervals for odds ratios in genome-wide association studies. *Biostatistics* 2008;**9**:621-34. Available from: https://doi.org/10.1093/biostatistics/kxn001.

[12] Bowden J, Smith GD, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;**44**:512-25. Available from: https://doi.org/10.1093/ije/dyv080.

[13] Bowden J, Smith GD, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. *Genet Epidemiol* 2016;**40**(4):304-14. Available from: https://doi.org/10.1002/gepi.21965.

[14] Hartwig FP, Smith GD, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int J Epidemiol* 2017;**46**:1985-98. Available from: https://doi.org/10.1093/ije/dyx102.

[15] Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An Open Access Resource for Identifying the Causes of a Wide Range of Complex Diseases of Middle and Old Age. *PLoS Med* 2015;**12**:1-10. Available from: https://doi.org/10.1371/journal.pmed.1001779.

[16] Zeng J, de Vlaming R, Wu Y, et al. Signatures of negative selection in the genetic architecture of human complex traits. *Nat Genet* 2018;**50**:746-53. Available from: https://doi.org/10.1038/s41588-018-0101-4.

[17] Bulik-Sullivan B, Finucane HK, Anttila V, et al. An atlas of genetic correlations across human diseases and traits. *Nat Genet* 2015;**47**:1236-41. Available from: https://doi.org/10.1038/ng.3406.

[18] Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018;**7**:e34408. Available from: https://doi.org/10.7554/elife.34408.

[19] Grotzinger AD, Rhemtulla M, de Vlaming R, et al. Genomic structural equation modelling provides insights into the multivariate genetic architecture of complex traits. *Nat Hum Behav* 2019;**3**:513-25. Available from: https://doi.org/10.1038/s41562-019-0566-x.

[20] Bulik-Sullivan BK, Loh PR, Finucane HK, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* 2015;**47**:291-5. Available from: https://doi.org/10.1038/ng.3211.

[21] International HapMap 3 Consortium. Integrating common and rare genetic variation in diverse human populations. *Nature* 2010;**467**:52-8. Available from: https://doi.org/10.1038/nature09298.

[22] Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018;**562**:203-9. Available from: https://doi.org/10.1038/s41586-018-0579-z.

[23] Zhao Q, Wang J, Hemani G, Bowden J, Small DS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. *Ann Stat* 2020;**48**. Available from: https://doi.org/10.1214/19-aos1866.

[24] Neale BM. GWAS RESULTS - GWAS round 2. http://www.nealelab.is/uk-biobank (July 2021, date last accessed).

[25] Sadreev II, Elsworth BL, Mitchell RE, et al. Navigating sample overlap, winner's curse and weak instrument bias in Mendelian randomization studies using the UK Biobank. *medRxiv*. doi:10.1101/2021.06.28.21259622, 2 July 2021, preprint: not peer reviewed. Available from: `https://doi.org/10.1101/2021.06.28.21259622`.

[26] Foley CN, Mason AM, Kirk PDW, Burgess S. MR-Clust: clustering of genetic variants in Mendelian randomization with similar causal estimates. *Bioinformatics* 2020;**37**:531-41. Available from: `https://doi.org/10.1093/bioinformatics/btaa778`.

[27] Morrison J, Knoblauch N, Marcus JH, Stephens M, He X. Mendelian randomization accounting for correlated and uncorrelated pleiotropic effects using genome-wide summary statistics. *Nat Genet* 2020;**52**:740-7. Available from: `https://doi.org/10.1038/s41588-020-0631-4`.

[28] Darrous L, Mounier N, Kutalik Z. Simultaneous estimation of bi-directional causal effects and heritable confounding from GWAS summary statistics. *medRxiv*. doi:10.1101/2021.06.28.21259622, 13 August 2021, preprint: not peer reviewed. Available from: `https://doi.org/10.1101/2020.01.27.20018929`.