Robust instrumental variable methods using multiple candidate instruments with application to Mendelian randomization

Stephen Burgess 1, *  Jack Bowden 2  Frank Dudbridge 1,3  Simon G. Thompson 1

1 Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

2 Integrative Epidemiology Unit, University of Bristol, Bristol, UK.

3 Department of Non-communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, UK.

June 14, 2016

Short title: Robust instrumental variable methods applied to Mendelian randomization.

*Address: Department of Public Health and Primary Care, Strangeways Research Laboratory, 2 Worts Causeway, Cambridge, CB1 8RN, UK. Telephone: +44 1223 748651. Correspondence to: sb452@medschl.cam.ac.uk.
Abstract

Mendelian randomization is the use of genetic variants to make causal inferences from observational data. The field is currently undergoing a revolution fuelled by increasing numbers of genetic variants demonstrated to be associated with exposures in genome-wide association studies, and the public availability of summarized data on genetic associations with exposures and outcomes from large consortia. A Mendelian randomization analysis with many genetic variants can be performed relatively simply using summarized data. However, a causal interpretation is only assured if each genetic variant satisfies the assumptions of an instrumental variable. To provide some protection against failure of these assumptions, we develop two extensions to methods for instrumental variable analysis using: i) robust regression and ii) the penalization of weights from candidate instruments with heterogeneous causal estimates. Results from a wide variety of methods, including the recently-proposed MR-Egger and median-based methods, are compared in an extensive simulation study. We demonstrate that two methods, robust regression in an inverse-variance weighted method and a simple median of the causal estimates from the individual variants, have considerably improved Type 1 error rates compared with conventional methods in a wide variety of scenarios when up to 30% of the genetic variants are invalid instruments. While the MR-Egger method gives unbiased estimates when its assumptions are satisfied, these estimates are less efficient than those from other methods and are highly sensitive to violations of the assumptions. Methods that make different assumptions should be used routinely to assess the robustness of findings from applied Mendelian randomization investigations with multiple genetic variants.

Keywords: Mendelian randomization, instrumental variables, robust methods, summarized data, aggregated data, MR-Egger.
1 Introduction

There are two broad approaches for obtaining causal inferences from observational data [1, 2]. The first is to make an assumption that the missing data (the unobserved potential outcomes [3]) can be modelled as a function of the measured data (“no unmeasured confounding”) [1]. The second approach is to exploit quasi-randomization; that is, a variable in the dataset can be treated as if it has been allocated at random [5]. A quasi-randomized variable is usually a factor that is external to the system under investigation, for example a change in cigarette tax when studying tobacco consumption as the exposure.

An instrumental variable (denoted $Z$) is a randomized or quasi-randomized variable that can be used to estimate the causal effect of an exposure (or risk factor, denoted $X$) on an outcome ($Y$) in the presence of arbitrary unmeasured confounding [6]. An instrumental variable satisfies three assumptions:

i) association with the exposure: $Z \perp \!\!\!\!\perp X$;

ii) independence from confounders (denoted $U$) of the exposure–outcome association: $Z \perp \!\!\!\!\perp U$;

iii) independence from the outcome conditional on the exposure and confounders: $Z \perp \!\!\!\!\perp Y|X, U$.

The assumptions imply that an instrumental variable cannot have a direct effect on the outcome, but instead any effect is mediated via the exposure (this is known as the exclusion restriction assumption) [7]. A directed acyclic graph illustrating these assumptions is given as Figure 1.

The instrumental variable assumptions are restrictive and often unrealistic in practice. One way of assessing whether these assumptions are satisfied is to compare the causal estimates from several proposed instrumental variables [8]. If the instrumental variable assumptions are satisfied, and under additional parametric assumptions (sufficient conditions are linearity of the instrumental variable–exposure, instrumental variable–outcome and exposure–outcome relationships with no effect heterogeneity), the same causal parameter is estimated by each of the instrumental variables [9]. In this paper, we use the term ‘candidate instrument’ to describe a variable that is hypothesized to satisfy the instrumental variable assumptions, without prejudicing whether the assumptions are satisfied or not.

A context in which there are often multiple candidate instruments that may plausibly satisfy the instrumental variable assumptions is Mendelian randomization [10, 11], the use of genetic variants as instrumental variables [9, 12]. For complex (i.e. polygenic and multifactorial) exposures, such as body mass index [13] or blood pressure [14], many associated genetic variants have been discovered in genome-wide association studies. A recent development in Mendelian randomization is the availability of summarized data [15]. These data comprise the associations (beta-coefficients and standard errors) of genetic variants with the exposure and with the outcome estimated
from univariable regression models. Such associations estimated in large sample sizes have been made publicly available for download by many consortia; examples include associations with glycaemic traits from the Meta-Analyses of Glucose and Insulin-related traits Consortium [16] and with schizophrenia and other psychiatric disorders from the Psychiatric Genomics Consortium [17]. Instrumental variable methods using summarized data have been recently developed, and include an inverse-variance weighted method [18], and two robust methods: MR-Egger [19] and a median-based method [20]; a robust method is defined here as one that can provide reasonable estimates under weaker assumptions than a conventional approach that assumes all candidate instruments are valid.

In this paper, we consider robust methods for causal inference using multiple instrumental variables, focusing on those that can be implemented using summarized data for uncorrelated candidate instruments. Although these robust methods have good theoretical properties, there are several issues particularly with the MR-Egger method, such as low power in realistic scenarios [20]. Additionally, the MR-Egger and median-based methods make different assumptions for consistent estimation of the causal effect. We seek to develop methods that provide reasonable estimates under the assumptions of either the MR-Egger or the median-based method.

In Section 2, we describe the inverse-variance weighted method and review the existing literature on robust methods, in particular the median-based and MR-Egger methods. We introduce two extensions to these methods: robust regression, and penalization of weights. In Section 3 we perform a simulation analysis to compare estimates from these robust methods with respect to bias and coverage properties when some of the candidate instruments do not satisfy the instrumental variable assumptions. Parameters in the simulation analysis are chosen to reflect a typical Mendelian randomization investigation. In Section 4 we show how these methods perform in an applied analysis of the causal effect of body mass index on the risk of schizophrenia. We conclude by discussing the results of this paper, and the potential for future developments (Section 5).

2 Methods

Existing robust methods for causal inference using instrumental variables have taken two different approaches. The first approach (which includes the median-based method) is to assume that some, but not all, of the candidate instruments satisfy the instrumental variable assumptions. The second approach (which includes the MR-Egger method) allows all of the candidate instruments not to be valid instrumental variables, but assumes that the set of variants satisfies a weaker assumption.

We assume throughout that the parametric assumptions of linearity with no effect heterogeneity hold for the causal exposure–outcome relationship, and for the instrumental variable–exposure and instrumental variable–outcome associations for
all instruments $Z_j$, $j = 1, \ldots, J$:

\[
E(X|Z_j = z, U = u) = \beta_{X0j} + \beta_{Xj} z + \beta_{XU} u
\]

\[
E(Y|Z_j = z, U = u) = \beta_{Y0j} + \beta_{Yj} z + \beta_{YU} u \quad \text{for } j = 1, \ldots, J
\]

\[
E(Y|do(X = x)) = \theta_0 + \theta x
\]

where $U$ is an unmeasured confounder, $do(X = x)$ is the do-operator of Pearl meaning that the value of the exposure is set to $x$ by intervention [21], and the causal effect parameter $\theta = \frac{\beta_{Yj}}{\beta_{Xj}}$ for all valid instruments. Issues relating to these parametric assumptions are left to the discussion. Software code for implementing all the methods used in this paper is provided in the Web Appendix.

### 2.1 Median-based method

The median-based method is motivated by the robustness of the median to outlying values. The median has a 50% breakdown point, meaning that median of the causal estimates obtained using each of the candidate instruments individually is a consistent estimator of the causal effect (as the sample size $N$ tends to infinity) provided that at least half of the candidate instruments satisfy the instrumental variable assumptions [22]. The simple median estimator is calculated here as the median of the ratio (or Wald [23]) estimates $\hat{\theta}_j$, where $\hat{\theta}_j = \frac{\hat{\beta}_{Yj}}{\hat{\beta}_{Xj}}$ is the ratio of the beta-coefficients from univariable regressions of the outcome on the $j$th candidate instrument ($\hat{\beta}_{Yj}$) and of the exposure on the $j$th candidate instrument ($\hat{\beta}_{Xj}$).

A weighted median of these causal estimates can also be considered to account for differences in the precision of estimates [20]. We assume that candidate instruments are ordered by the magnitude of their estimates (so that $\hat{\theta}_1 < \hat{\theta}_2 < \ldots < \hat{\theta}_J$), and define the weight $w_j$ for estimate $j$ as:

\[
w_j = \frac{\hat{\beta}_{Xj}^2}{\text{se}(\hat{\beta}_{Yj})^2} \left/ \sum_{l=1}^{J} \frac{\hat{\beta}_{Xl}^2}{\text{se}(\hat{\beta}_{Yl})^2} \right.
\]

(2)

These weights are the inverse-variance weights, where the variance of the ratio estimate is approximated as the first term from a delta method expansion [24]:

\[
\text{var}(\hat{\theta}_j) = \frac{\text{se}(\hat{\beta}_{Yj})^2}{\hat{\beta}_{Xj}^2}.
\]

(3)

Weights are normalized so that their sum is equal to 1. The weighted median estimate is the weighted average of the $k$th and $(k + 1)$th ratio estimates, where $k$ is the largest integer such that the cumulative weight up to and including the $k$th estimate ($s_k = \sum_{j \leq k} w_j$) is less than 0.5. The estimate can be calculated by interpolation.
between the \( k \)th and the \((k + 1)\)th ratio estimates:

\[
\hat{\theta}_{WM} = \hat{\theta}_k + (\hat{\theta}_{k+1} - \hat{\theta}_k) \times \frac{0.5 - s_k}{s_{k+1} - s_k}.
\]  

(4)

The simple median estimate is the same as the weighted median estimate when all the weights are equal. For the weighted median estimate, the consistency assumption is that at least 50% of the weight in the analysis comes from valid instrumental variables.

Standard errors for the simple and weighted median estimates can be calculated by bootstrapping; confidence intervals are obtained using a bootstrapped standard error and a normal approximation \([20]\). Software code illustrating this procedure is provided in the Web Appendix.

### 2.2 Inverse-variance weighted and MR-Egger methods

The MR-Egger method is performed by a weighted linear regression of the associations of the candidate instruments with the outcome \((\hat{\beta}_{Yj})\) on the associations of the candidate instruments with the exposure \((\hat{\beta}_{Xj})\):

\[
\hat{\beta}_{Yj} = \theta_{0E} + \theta_{1E} \hat{\beta}_{Xj} + \epsilon_{Ej}, \text{ weights } = \text{se}(\hat{\beta}_{Yj})^{-2}.
\]  

(5)

The genetic variants are coded such that all their associations with the exposure are positive (the sign of each genetic association with the exposure can be chosen arbitrarily by varying which allele is regarded as the reference allele, provided that the association with the outcome is expressed with reference to the same allele). Weights are the inverse-variance weights taken from univariable regressions of the outcome on each candidate instrument. If the intercept \(\theta_{0E}\) in the regression model \([5]\) is estimated, then the slope estimate \(\hat{\theta}_{1E}\) is the MR-Egger estimate of the causal effect \(\theta\) \([19]\). If the intercept is fixed at zero, then the slope estimate \(\hat{\theta}_{1E}\) is the inverse-variance weighted (IVW) estimate \([25]\). This same estimate can be obtained by inverse-variance weighted meta-analysis of the ratio estimates \(\hat{\theta}_j\) using the variance expression in equation \([3]\) \([18]\). Additionally, the IVW estimate is the same as the two-stage least squares estimate that can be calculated using individual-level data \([26]\) (assuming, as throughout, that the candidate instruments are uncorrelated).

### 2.3 Conditions for consistency of the IVW and MR-Egger estimates

Under linearity assumptions, the association of a candidate instrument with the outcome \(\beta_{Yj}\) decomposes into a direct effect \(\alpha_j\) and an indirect effect that corresponds to the causal effect of the exposure on the outcome \(\theta\) multiplied by the association of the candidate instrument with the exposure \((\beta_{Xj})\) \([27]\):

\[
\beta_{Yj} = \alpha_j + \theta \beta_{Xj}.
\]  

(6)

(Hats are omitted as this decomposition is expressed in terms of the underlying parameters.) The term ‘pleiotropy’ refers to a genetic variant having associations with
more than one risk factor on different causal pathways [12]. A pleiotropic genetic variant is typically not a valid instrumental variable. In this decomposition, genetic variant $j$ is pleiotropic if $\alpha_j \neq 0$, and $\alpha_j$ is referred to as the pleiotropic effect. The decomposition is illustrated in Figure 2 (the genetic variant is denoted $G_j$ rather than $Z_j$ to emphasize that it is not a valid instrument if $\alpha_j \neq 0$).

For each instrument $j$, the ratio causal estimate is equal to:

$$\frac{\hat{Y}_j}{\hat{X}_j} \xrightarrow{N \to \infty} \frac{\beta_{Yj} \alpha_j + \theta \beta_{Xj}}{\beta_{Xj}} = \theta + \frac{\alpha_j}{\beta_{Xj}},$$

(7)

where $N$ is the sample size. A valid instrumental variable has no direct effect on the outcome ($\alpha_j = 0$), so the ratio estimate for a valid instrument is consistent for the causal effect $\theta$. The IVW estimate is a weighted average of the ratio estimates, and so is also consistent for the causal effect when all instruments are valid.

More generally, the IVW estimate is:

$$\frac{\sum_j \hat{Y}_j \beta_{Xj} \text{se}(\hat{Y}_j)^{-2}}{\sum_j \beta_{Xj}^2 \text{se}(\hat{Y}_j)^{-2}} \xrightarrow{N \to \infty} \theta + \frac{\sum_j \alpha_j \beta_{Xj} \text{se}(\hat{Y}_j)^{-2}}{\sum_j \beta_{Xj}^2 \text{se}(\hat{Y}_j)^{-2}}.$$  

(8)

Therefore the asymptotic bias of the IVW estimate is zero if $\sum_j \alpha_j \beta_{Xj} \text{se}(\hat{Y}_j)^{-2} = 0$; otherwise the IVW estimate is a biased estimate of the causal effect.

The MR-Egger estimate is a consistent estimate of the causal effect under the condition that the pleiotropic effects of the candidate instruments $\alpha_j$ are uncorrelated with the associations of the candidate instruments with the exposure $\beta_{Xj}$ [19]. This is referred to as the InSIDE assumption (INstrument Strength Independent of Direct Effect). Specifically, we require the weighted covariance $\text{cov}_w(\alpha, \beta_{X})$ to be zero:

$$\text{cov}_w(\alpha, \beta_{X}) = \frac{\sum_j (\alpha_j - \bar{\alpha}_w)(\beta_{Xj} - \bar{\beta}_{Xw}) \text{se}(\hat{Y}_j)^{-2}}{\sum_j \text{se}(\hat{Y}_j)^{-2}} = 0$$

(9)

where $\bar{\beta}_{Xw}$ is the weighted mean of the $\beta_{Xj}$, $\bar{\alpha}_w$ is the weighted mean of the $\alpha_j$, and bold symbols represent vectors across the candidate instruments. The slope coefficient from the weighted regression analysis is:

$$\hat{\theta}_{1E} = \frac{\text{cov}_w(\beta_{Y}, \beta_{X})}{\text{var}_w(\beta_{X})} \xrightarrow{N \to \infty} \frac{\text{cov}_w(\beta_{Y}, \beta_{X})}{\text{var}_w(\beta_{X})} = \frac{\text{cov}_w(\alpha, \beta_{X})}{\text{var}_w(\beta_{X})} + \theta \frac{\text{cov}_w(\beta_{X}, \beta_{X})}{\text{var}_w(\beta_{X})}$$

(10)

which is equal to $\theta$ under the InSIDE assumption, where $\text{var}_w$ is the weighted variance function.

Under the InSIDE assumption, the intercept term in the MR-Egger analysis can be interpreted as the average pleiotropic effect of the candidate instruments included in the analysis. The intercept term is zero when there is balanced pleiotropy (that is,
the weighted average pleiotropic effect \( \bar{\alpha}_w = \frac{\sum_j \alpha_j \text{se}(\hat{\beta}_{Yj})^{-2}}{\sum_j \text{se}(\hat{\beta}_{Yj})^{-2}} \) is zero) and the InSIDE assumption is satisfied. In this case, the MR-Egger and IVW estimates will coincide, and the IVW estimate will also be consistent; these two conditions imply that the bias term for the IVW estimate \( \sum_j \alpha_j \beta X_j \text{se}(\hat{\beta}_{Yj})^{-2} \) is zero.

If the intercept term in the MR-Egger analysis differs from zero, then either the InSIDE assumption is violated, or the average pleiotropic effect differs from zero (referred to as directional pleiotropy). In either case, the instrumental variable assumptions are violated for at least one of the candidate instruments. However, provided that the InSIDE assumption holds, the MR-Egger estimate will still be consistent for the causal effect even in the case of directional pleiotropy. The statistical test of whether the intercept term in MR-Egger differs from zero – an assessment of evidence for either directional pleiotropy or violation of the InSIDE assumption – is referred to as the MR-Egger intercept test.

2.4 Finite-sample and population versions of the InSIDE assumption

As a technical aside, in the original description of MR-Egger, the InSIDE assumption was presented as the independence between the distribution of pleiotropic effects and the distribution of associations of the candidate instruments with the exposure [19]. This is a population version of the InSIDE assumption, and requires the candidate instruments to be conceptualized as being sampled from a population of candidate instruments. Consistency of the MR-Egger estimate under the population version of the InSIDE assumption requires the number of instruments to tend to infinity. In contrast, the version of the InSIDE assumption used in the proofs of consistency above is that the weighted covariance between the pleiotropic effects and associations of the candidate instruments with the exposure is zero for the particular set of candidate instruments in the analysis; this is a finite-sample version of the InSIDE assumption. This distinction also affects the definition of balanced and directional pleiotropy: in the population version, balanced pleiotropy is defined as the weighted mean of the distribution of pleiotropic effects equalling zero; in the finite-sample version, balanced pleiotropy is defined as the weighted mean of the pleiotropic effects equalling zero for the candidate instruments included in the analysis.

2.5 Fixed- and random-effects

The standard error from a weighted regression analysis, such as used in the IVW and MR-Egger methods above, can be obtained in different ways. In a fixed-effect analysis, the candidate instruments are all assumed to identify the same causal effect, and the standard error is based solely on the inverse-variance weights. This can be achieved in a regression model by fixing the residual standard error to be 1; in most statistical software packages, this is implemented by dividing the reported standard error of the regression coefficient by the estimate of the residual standard error [28]. Alternatively, the same standard error estimate can be obtained by a fixed-effect
IVW meta-analysis of the ratio estimates as described above [25]. However, if the ratio estimates are more variable than would be expected according to their standard errors (over-dispersion), then a random-effects analysis may be preferred [26]. This can be achieved by reporting the standard errors of the regression coefficients with no correction for the residual standard error.

In the MR-Egger method, over-dispersion (that is, excess heterogeneity in causal estimates beyond what would be expected by chance alone) is permitted (as the candidate instruments are allowed to have pleiotropic effects), and so a fixed-effect analysis would be inappropriate. Similarly in the IVW method, a fixed-effect analysis would be inappropriate if the ratio estimates of different candidate instruments were heterogeneous, for example under balanced pleiotropy. In this paper, we perform random-effects analyses allowing for (multiplicative) over-dispersion of estimates, but not permitting under-dispersion, as under-dispersion for uncorrelated candidate instruments has no natural interpretation other than being a chance finding. Hence, we divide the reported standard errors from all regression analyses by the minimum of 1 and the estimated residual standard error. Software code illustrating this correction is provided in the Web Appendix.

2.6 Motivation: robustness to either consistency assumption

The MR-Egger regression has a 100% breakdown point in the sense that all of the candidate instruments can violate the instrumental variable assumptions by having direct effects on the outcome ($\alpha_j \neq 0$), provided that the InSIDE assumption is satisfied. However, it relies on the InSIDE assumption being satisfied for the complete set of candidate instruments. In contrast, in the simple median method, up to 50% of candidate instruments can violate the instrumental variable assumptions arbitrarily. It would be worthwhile to develop a method that gives robust estimates if either of these two assumptions holds. We therefore seek extensions to the methods above to provide robustness to the influence of candidate instruments that yield heterogeneous estimates. We propose two novel approaches to achieve this: 1) robust regression and 2) penalization of weights. Both approaches downweight the contribution to the causal estimate of candidate instruments with heterogeneous ratio estimates.

2.7 Robust regression

Several methods have been proposed for performing robust regression (that is, regression with greater than a 0% breakdown point) [29]. Here, we use an MM-estimation approach as described by Koller and Stahel [30]. Each of the letter Ms refers to a “maximum likelihood type” maximization step. Briefly, the method proceeds by finding a robust S-estimate (“scale-type estimate”) that minimizes an M-estimate of the scale of the residuals (the first M in the method’s name). The estimated scale is then held constant whilst a close-by M-estimate of the parameters is located (the second M) [31]. This provides robustness both to outliers and to data points with high leverage. Further robustness is provided by using Tukey’s bisquare objective function [32]: instead of minimizing the sum of squared residuals, we minimize the sum of a function of the residuals that is capped at a maximum value for each residual. This means
that an outlier in the regression analysis has the same contribution to the objective function no matter how extreme the outlier is.

If the objective function of the standardized residuals \( r_j \) in the regression is \( \sum_j \rho(r_j) \), then ordinary least squares regression minimizes the sum of the square of the residuals, \( \rho(r_j) = r_j^2 \). In Tukey’s bisquare objective function,

\[
\rho(r_j) = \begin{cases} 
\frac{c^2}{6} \left(1 - \left(\frac{r_j}{c}\right)^2\right)^3 & \text{if } |r_j| < c \\
\frac{c^2}{6} & \text{if } |r_j| \geq c.
\end{cases}
\] (11)

The value of the tuning parameter \( c \) is chosen as 1.548 to provide a high breakdown point in the S-estimation step, and as 4.685 to provide an efficient estimator in the M-estimation steps. This method for robust regression is the default choice implemented by the \texttt{lmrob} command in the R package \texttt{robustbase} [33].

### 2.8 Penalization of weights

Another way of providing additional robustness is to penalize the weights of candidate instruments with heterogeneous ratio estimates in the weighted regression model. This could be achieved in many ways; we propose an approach using Cochran’s Q statistic as a measure of heterogeneity:

\[
Q = \sum_j Q_j = \sum_j \text{var}(\hat{\theta}_j)^{-1}(\hat{\theta}_j - \hat{\theta})^2
\] (12)

where \( \hat{\theta} \) is here taken as the IVW estimate. The Q statistic has an approximate \( \chi^2_{J-1} \) distribution under the null hypothesis that all candidate instruments satisfy the instrumental variable assumptions; the components of the Q statistic for each candidate instrument \( (Q_j) \) approximately have \( \chi^2_1 \) distributions. So as not to distort the majority of weights, we propose penalization using the one-sided upper p-value (denoted \( q_j \)) on a \( \chi^2_1 \) distribution corresponding to \( Q_j \), by multiplying the weight \( \text{se}((\hat{\beta}_Y)_{j})^{-2} \) by \( \min(1, 20q_j) \). The same downweighting factor has previously been used for weights in the median-based method to give a penalized weighted median estimate [20].

For the median-based methods, we replace the IVW estimate by the relevant median estimate. For MR-Egger, we consider a Q statistic equivalent to the residual sum of squares from the weighted regression, which has an approximate \( \chi^2_{J-2} \) distribution if the MR-Egger regression model is correct [33]:

\[
Q = \sum_j Q_j = \sum_j \sigma^2_{Yj}(\hat{\beta}_{Yj} - \hat{\theta}_0E - \hat{\theta}_1E\hat{\beta}_X) \]
(13)

### 3 Simulation study

We perform a simulation study to compare the bias and coverage properties of estimates from different methods:
• standard linear regression without and with an intercept term using inverse-variance weights as in equation (3) – this is equivalent to the IVW and MR-Egger methods respectively;
• robust linear regression without and with an intercept term using inverse-variance weights;
• standard linear regression without and with an intercept term using penalized inverse-variance weights;
• robust linear regression without and with an intercept term using penalized inverse-variance weights;
• simple, weighted, and penalized weighted median estimates.

We investigate whether these methods give reasonable inferences (in particular, maintain nominal Type 1 error rates under the causal null hypothesis \[ \theta = 0 \], but have reasonable power under the alternative) in realistic scenarios. Robust regression is implemented using the `lmrob` command from the `robustbase` package in R [35] with the `method = "MM"` option [33].

### 3.1 Data-generating model

The data-generating model for the simulation study is as follows:

\[
U_i = \sum_{j=1}^{J} \phi_j G_{ij} + \epsilon_{Ui} \tag{14}
\]

\[
X_i = \sum_{j=1}^{J} \gamma_j G_{ij} + U_i + \epsilon_{Xi}
\]

\[
Y_i = \sum_{j=1}^{J} \alpha_j G_{ij} + \theta X_i + U_i + \epsilon_{Y_i}
\]

\[G_{ij} \sim \text{Binomial}(2, 0.3)\] independently for all \(j = 1, \ldots, J\)

\[\epsilon_{Ui}, \epsilon_{Xi}, \epsilon_{Y_i} \sim \mathcal{N}(0, 1)\] independently

for participants indexed by \(i = 1, \ldots, N\), and candidate instruments indexed by \(j = 1, \ldots, J\). The candidate instruments \(G_j\) are simulated to be equivalent to genetic variants that are single nucleotide polymorphisms in Hardy–Weinberg equilibrium with minor allele frequency 0.3. The variable \(U\) is a confounder in the relationship between the exposure and the outcome, and is assumed to be unmeasured. The error terms \(\epsilon_{Ui}, \epsilon_{Xi},\) and \(\epsilon_{Y_i}\) were each drawn independently from standard normal distributions. The causal effect of the exposure on the outcome was either \(\theta = 0\) (null causal effect) or \(\theta = 0.1\) (positive causal effect). The effects of the candidate instruments on the exposure (\(\gamma_j\)) were drawn from a uniform distribution between 0.03 and 0.1. The direct effects of a candidate instrument (genetic variant) on the outcome (\(\alpha_j\)) and the effects of the candidate instruments on the confounder (\(\phi_j\))
were set to zero if the candidate instrument was a valid instrumental variable; for candidate instruments that were invalid instrumental variables:

- In Scenario 2 (direct effects average to zero – balanced pleiotropy, population InSIDE satisfied), the $\alpha_j$ parameters were drawn from a uniform distribution between $-0.1$ and $0.1$, and the $\phi_j$ were taken as $0$.

- In Scenario 3 (direct effects do not average to zero – directional pleiotropy, population InSIDE satisfied), the $\alpha_j$ parameters were drawn from a uniform distribution between $0$ and $0.1$, and the $\phi_j$ were taken as $0$.

- In Scenario 4 (direct effects operate via confounder and hence do not average to zero – directional pleiotropy, InSIDE not satisfied), the $\phi_j$ parameters were drawn from a uniform distribution between $-0.1$ and $0.1$, and the $\alpha_j$ were taken as $0$.

In Scenario 1, all candidate instruments are taken to be valid instruments. In Scenarios 2 to 4, the probability of being an invalid instrumental variable was set to 0.1, 0.2, and 0.3.

A total of 10,000 simulated datasets were generated for $N = 40,000$ participants and $J = 25$ candidate instruments. A ‘two-sample’ setting was assumed in which associations of the candidate instruments with the exposure were estimated in the first $N/2$ participants, and associations with the outcome in the second $N/2$ participants. Results obtained in a one-sample setting in which the associations with the exposure and with the outcome are obtained in the same individuals are given in the Web Appendix. Only the summarized data, that is the estimated univariable associations of the candidate instruments with the exposure and with the outcome, and their standard errors, were used by the analysis methods. The average proportion of variance in the exposure explained by the candidate instruments ($R^2$ statistic) was 2.5% (2.8% in Scenario 4), and the average F statistic was 20.5 (23.3 in Scenario 4).

### 3.2 Results

Results from the simulation study are provided in Table 1 (Scenario 1 only, all methods), Table 2 (Scenarios 2-4, weights not penalized), and Table 3 (Scenarios 2-4, penalized weights). Table 1 displays the mean estimate across simulations, standard deviation of estimates, mean standard error of estimates, and the empirical power to detect a causal effect (the proportion of simulations where the 95% confidence interval [estimate ± 1.96 standard errors] excluded the null). With a null causal effect, power to detect a causal effect is the same as the Type I error rate, and the expected power is 5%. In Tables 2 and 3, the mean standard error of estimates is omitted (the pattern of results for the mean standard error was similar to that in Scenario 1 except as noted below). In some of the simulations, the robust regression method did not report a standard error (less than 1% in all cases, except up to 2.5% for the robust method with an intercept in Scenario 4); the number of simulations that failed to report a standard error is given in Table 1 for Scenario 1, and in Web Table A1 for other scenarios. Simulations were not excluded from the results if a standard error
was not reported (except for the calculation of the mean standard error); power calculations include these simulations as if the standard error estimate is infinite. The Monte Carlo standard error (the uncertainty due to the limited number of simulations considered) for the power was 0.2% with a null effect, and between 0.2% and 0.5% with a positive causal effect.

**Scenario 1 (Table 1):** When all candidate instruments were valid instruments, all methods provided unbiased estimates under the null, with Type 1 error rates close to or below the nominal significance level of 5%. The standard deviation of estimates was slightly below the mean standard error of estimates for all methods, with differences most marked for the median-based methods. This difference suggests that methods may be slightly conservative in their inferences. In terms of precision of the causal estimate, regression methods without an intercept (including the IVW method) were the most precise, followed closely by the median-based methods, while regression methods with an intercept (including the MR-Egger method) were the least precise. Differences in precision between the standard, robust and penalized methods were slight.

With a positive causal effect, differing precisions of the causal estimate were also evidenced by the marked differences in power to detect a causal effect. The power for the regression methods including an intercept term was barely above 5%. While precision of the causal estimate for the IVW method depends on the proportion of variance in the exposure explained by the candidate instruments, precision of the causal estimate for MR-Egger depends on the variability between the instrument–exposure associations [36]. If all candidate instruments have exactly the same magnitude of association with the exposure, then the MR-Egger estimate is undefined. The MR-Egger estimate will always be less precise than the IVW estimate, but the difference in precision will depend on whether the instrument–exposure associations for different candidate instruments are similar to each other or not.

While there was some attenuation towards the null with a positive causal effect for all the methods (except for the simple median method) due to uncertainty in the genetic associations with the exposure, this was minimal for the IVW and other methods with no intercept, but substantial for the MR-Egger and other methods with an intercept. This attenuation is a known phenomenon called finite-sample bias (also known as weak instrument bias [37]). Bias in the two-sample setting acts towards the null [38] and is related to regression dilution bias [39]; it arises due to measurement error in the independent variable in a regression model. Relative bias of the IVW estimate is around $1/F$, where $F$ is the expected value of the $F$ statistic from regression of the exposure on the IVs (here, $1/F \approx 1/20 = 5\%$, similar to the observed attenuation in the mean IVW estimates) [40]; whereas attenuation of the MR-Egger estimate is approximately equal to the $I^2$ statistic from meta-analysis of the weighted associations with the exposure $\hat{\beta}_X \cdot \text{se}(\hat{\beta}_X)^{-1}$ with standard errors $\text{se}(\hat{\beta}_X) \cdot \text{se}(\hat{\beta}_Y)^{-1}$ [36]. The $I^2$ statistic is large when the genetic variants have a wide spread of associations with the exposure or their associations are precisely estimated, and small when their associations with the exposure are imprecisely measured or all similar. In the simulation, the average value of the $I^2$ statistic was 60.1%. This bias can be corrected using the Simulation Extrapolation (SIMEX) method [41, 36], although this was not
computationally feasible in the simulation setting. While measurement error in the exposure can lead to inflation of the intercept term in the MR-Egger method, in this case the 95% confidence interval for the intercept term excluded zero for MR-Egger in 4.7% of simulations – close to the expected nominal 5% level, indicating that over-rejection of the null hypothesis for the MR-Egger intercept test was not evident in this example.

Scenario 2, 3 and 4, non-penalized weights (Table 2): Mean estimates in Scenario 2 (balanced pleiotropy, InSIDE satisfied) were unbiased with a null causal effect for all methods. With a positive causal effect, mean estimates were similar to those in Scenario 1: close to unbiased for most methods, but with severe attenuation for regression methods with an intercept term. However, there were marked differences in the precision of estimates compared with Scenario 1. Out of previously proposed methods, estimates from the median-based methods were more precise than those from the IVW method, although this did not translate into greater power with a positive causal effect when only 10% of candidate instruments were not valid instruments. However, the greatest power was obtained by the robust regression method with no intercept. Although the power of the MR-Egger method and other regression methods with an intercept was low, the use of robust regression did reduce the standard deviation and mean standard error of estimates.

Scenario 3 (directional pleiotropy, InSIDE satisfied) demonstrates the value of the MR-Egger and related methods estimating an intercept for providing robust inferences under the InSIDE assumption. While estimates from other methods (particularly the IVW method) were biased under the null, mean estimates from regression methods with an intercept term were close to unbiased, and Type 1 error rates were close to nominal levels. But again, these methods were unable to identify the presence of a causal effect with reasonable power, and mean estimates were substantially attenuated. More seriously, in Scenario 4 (directional pleiotropy, InSIDE not satisfied), the MR-Egger method performed much worse than the IVW method, with mean estimates far more biased and larger Type 1 error rates. While there was some improvement using robust regression with an intercept term when there were few invalid instruments, there was still substantial bias and Type 1 error inflation, as well as even less precise estimates compared with the MR-Egger method when there were many invalid instruments. The MR-Egger and related methods are highly sensitive to the validity of the InSIDE assumption. As the InSIDE assumption is not testable, this is a major limitation of these methods.

In contrast, while the median-based methods and robust regression method without an intercept had bias in mean estimates and inflated Type 1 error rates, rates were substantially below those for the IVW method. In particular, Type 1 error rates were close to 10% or below for the simple median method in Scenario 4, and for the simple median and robust regression without an intercept methods in Scenario 3 with up to 20% invalid instruments. The weighted median method was particularly poor in Scenario 4; the data-generating mechanism meant that the invalid instruments received more weight in the analysis than the valid instruments as they had greater associations with the exposure (via an additional association with the confounder). The
median-based methods and robust regression without an intercept also had reasonable power to detect a causal effect when present.

Scenarios 2, 3 and 4, penalized weights (Table 3): The use of penalized weights generally led to more precise causal estimates, and Type 1 error rates were somewhat improved in Scenarios 3 and 4 for the IVW and weighted median methods. However, Type 1 error rates for the penalized methods generally exceeded nominal levels in all scenarios, especially when 20% or more candidate instruments were invalid. A particular cause for concern is the inflated Type 1 error rates in Scenario 2, which did not occur with unpenalized weights. The reason seems to be that the heterogeneity between the estimates from candidate instruments was underestimated, and hence there was underestimation of the uncertainty in the causal estimate. This highlights a danger that penalization of weights can lead to overconfidence in making inferences, as evidence that points in a different direction is downweighted in the analysis. Penalization of weights did not seem to be a worthwhile strategy for controlling Type 1 error rates in this simulation study.

Supplementary analyses: This simulation was repeated in a one-sample setting in which associations of the candidate instruments with the exposure and with the outcome were obtained in the same sample of 20,000 individuals for the methods using non-penalized weights. Results are displayed in Web Table A2 (Scenario 1) and Web Table A3 (Scenarios 2 to 4). Bias in the direction of the observational association was observed in all methods except for the simple median method (which remained unbiased in Scenarios 1 and 2). However, the bias of the MR-Egger method was greater and more severe than that of the IVW method: in Scenario 1 with a null causal effect, the mean estimates were 0.024 for the IVW method and 0.173 for the MR-Egger method, and Type 1 error rates were 6.8% and 27.2% respectively. The rejection rate of the MR-Egger intercept test was also inflated (23.5% with a null causal effect, 20.3% with a positive causal effect). The one-sample setting is another case where the MR-Egger method performs poorly.

The simulation was also repeated in a two-sample setting with only 10 candidate instruments, to observe whether the robust methods were able to operate well with fewer instruments to detect violations of the instrumental variables assumptions. Results for Scenarios 2 to 4 are presented in Web Table A4. Power to detect a causal effect was generally much lower, but otherwise similar results were observed.

Finally, Table 4 shows the proportion of datasets for the original simulation study rejecting the causal null using both the simple median and robust regression method with no intercept (robust IVW), and the empirical power of the MR-Egger intercept test for detecting directional pleiotropy and/or violations of the InSIDE assumptions. The combination of the simple median and robust IVW methods generally provided conservative inferences, with Type 1 error rates close to or below nominal levels except in Scenario 3 with 30% invalid inferences. This suggests that multiple robust methods
could be used as sensitivity analyses in practice to better control Type 1 error rates. The MR-Egger intercept test is a test of directional pleiotropy and/or violation of the InSIDE assumption: as expected, rejection rates were around 5% in Scenarios 1 and 2, and greater in Scenarios 3 and 4. This suggests that, even if the MR-Egger estimate is unreliable, the method may be useful for detecting in which cases the IVW method is likely to be biased.

[Table 4 should appear about here.]

4 Applied example: causal effect of body mass index on schizophrenia risk

As an applied example to illustrate the methods, we considered the causal effect of body mass index (BMI) on schizophrenia risk. Individuals with schizophrenia generally have higher incidence of obesity than the general population [42], although the relationship is thought to arise from the effect of anti-psychotic medicine on BMI (reverse causation) rather than as a causal effect of BMI [43]. We use 97 genetic variants previously demonstrated to be associated with BMI at a genome-wide level of significance by the Genetic Investigation of Anthropometric Traits (GIANT) consortium [44]. Associations with the exposure were taken from univariable linear regression analyses in up to 339,224 European-descent individuals from the GIANT consortium [44]; associations with the outcome were taken from univariable logistic regression analyses in around 9000 European-descent cases and 8000 controls from the Psychiatric Genomics Consortium [17]. The 97 genetic variants explain about 2.7% of the variance in BMI. Both sets of genetic associations have previously been made publicly available, and the association estimates can be obtained using the PhenoScanner tool at http://phenoscanner.medschl.cam.ac.uk/; they are also displayed visually in Figure 3. The graph indicates that there are several genetic variants that are clear outliers in their associations with schizophrenia, suggesting potential pleiotropy. The $I^2$ statistic for the weighted genetic associations with the exposure was 88.8%, suggesting that attenuation of the MR-Egger and other methods that estimate an intercept should not be severe.

[Figure 3 should appear about here.]

Estimates and 95% confidence intervals are provided in Table 5. Random-effects models were used in all analyses. Each estimate represents the log odds ratio for schizophrenia per 1 standard deviation increase in BMI. Although all estimates are compatible with the null, there is a wide variation in the standard errors of estimates. Using non-penalized weights, a similar pattern of results was seen as in the simulation analyses of Scenario 2: the robust method with no intercept giving the most precise estimate, followed by the median-based methods, with the MR-Egger method far behind. The use of penalized weights led to large improvements in precision for all except the median-based methods, indicating that although penalization of weights did not seem to add robustness to results in the simulation study, it may have a role
in improving the precision of results in cases like this where there are genetic variants that are clear outliers. In the IVW method, the use of penalized weights reduced the residual standard error from 2.14 to 1.12, only slightly above the value of 1 that would be expected in the absence of heterogeneity. In an applied setting, the genetic variants that are downweighted in the analysis should be examined for pleiotropy to determine whether their omission from the analysis is reasonable.

This applied example illustrates that in addition to providing additional confidence in findings from a conventional analysis, the methods introduced in this paper have the potential to improve the efficiency of Mendelian randomization estimates.

Table 5 should appear about here.

5 Discussion

In this paper, we have introduced two extensions to instrumental variable methods to downweight the influence of candidate instruments with heterogeneous causal estimates. A summary of the methods presented in this paper is provided in Table 6. Both the use of robust regression and of penalized weights reduce the sensitivity of methods to the influence of outlying variants, so that their use with the MR-Egger method should provide more robust estimates in large samples in the case that all candidate instruments satisfy the InSIDE assumption, or the case that most of the candidate instruments are valid instruments. However, the more relevant question to determine the practical usefulness of a method is whether it gives sensible results in realistic settings for finite samples.

Table 6 should appear about here.

While the robust and the penalized versions of MR-Egger have desirable theoretical properties, in our simulation study neither method was able to reliably detect the presence of a causal effect of moderate size with reasonable power. Additionally, both these and the original MR-Egger method were highly sensitive to violations of the InSIDE assumption. The MR-Egger intercept test was able to detect scenarios in which the IVW method gave biased estimates, although power was moderate at best. The two methods that had the best performance across a range of scenarios in terms of Type 1 error rate and power were the robust version of the IVW method and the simple median method. If other parameters were chosen in the simulation study, then different results might have been observed; for example, if candidate instruments had substantially different strengths (and validity of the candidate instruments did not depend on instrument strength, as in Scenario 4), then the weighted median method may have been preferable to the simple median method, and the loss of power in the MR-Egger method compared with the IVW method would have been less severe. Alternatively, if simulations were conducted in a scenario where 100% of the candidate instruments were invalid but they satisfied the InSIDE assumption, then the MR-Egger method would have fared better; likewise if the magnitude of the causal effect was greater (hence the MR-Egger method would have had improved power to detect a causal effect), or if the sample size for the genetic associations with the exposure
increased (hence the MR-Egger estimates would have been less attenuated). However, while we would hesitate to extrapolate too strongly from a single set of simulations, the robust version of the IVW method seems to be a worthwhile sensitivity analysis method in addition to other robust methods previously proposed (such as simple and weighted median, and MR-Egger [45]). The use of penalized weights may be worthwhile to improve precision if a small number of candidate instruments have clearly heterogeneous causal estimates (as demonstrated in the applied example), but the approach is unlikely to lead to robust inferences if several candidate instruments are not valid.

5.1 Linearity and homogeneity assumptions

In the specification of the analysis models, we have assumed linearity and homogeneity (no effect modification) of the causal effect of the exposure on the outcome, and of the associations of the candidate instruments with the exposure and with the outcome. These assumptions are not necessary to identify a causal effect; weaker assumptions can be made [46] (such as monotonicity of the causal effect [47] or a weaker version of the homogeneity assumption for the causal effect [48, 49]). If the linearity and homogeneity assumptions are violated, then the causal estimate using a single instrumental variable is a valid test of the null hypothesis that the exposure does not have a causal effect on the outcome [9]; this also applies to the causal estimate from the IVW method using multiple instruments, as this is a linear combination of the causal estimates from the individual instruments [25]. Hence, even when the linearity and homogeneity assumptions are violated, the methods proposed in this paper can still be used for the assessment of the causal null hypothesis (does the exposure have a causal effect on the outcome?), even if the estimate does not have a literal interpretation [50].

Additionally, while the linearity and homogeneity assumptions are stringent, genetic variants tend to have small effects on the exposure and outcome. This means that linearity and homogeneity may not be unreasonable assumptions in an applied Mendelian randomization investigation. Linearity and homogeneity in the genetic associations are not required across the whole distribution of the exposure and the outcome, but simply in the range of values predicted by the genetic variants.

5.2 Alternative robust methods

Several other methods have been developed for robust estimation using instrumental variables. Kang et al. proposed a method based on penalized regression for detecting and accounting for invalid instruments that provides a consistent estimate of causal effect if at least 50% of the candidate instruments are valid using L1-penalization to downweight the contribution to the analysis of candidate instruments that have heterogeneous causal estimates [27]. Han proposed a similar penalized estimator within the generalized method of moments framework, again with a 50% breakdown level [22]. Kolesár et al. proposed a method within the framework of k-class estimators with a 100% breakdown level under the InSIDE assumption [51]. The first two methods are similar to the median-based methods discussed here, and the final method is similar
to the MR-Egger method. A method similar to the penalization approach introduced here was proposed by Windmeijer et al. [52]. This consists of testing the homogeneity of the causal estimates from different sets of candidate instrumental variables using Hansen’s overidentification test, and reporting a causal estimate based on candidate instruments whose causal estimates are mutually similar. Each of these methods requires individual-level data; we look forward to the development of versions of these methods for summarized data, which would increase their applicability to applied Mendelian randomization investigations.

5.3 Conclusion

We have shown that it is difficult to find methods that give robust causal inferences with invalid instruments. Even in the examples with moderate numbers of invalid instruments considered in this paper, all methods had inflated Type 1 error rates in at least one scenario. Nevertheless, although the methods we have proposed are far from perfect, they have much improved Type 1 error rates compared with the conventional IVW method and the recently introduced MR-Egger method in scenarios where the InSIDE assumption fails to hold.

We have demonstrated that using multiple methods for instrumental variable analysis (particularly methods that provide consistent estimates under different assumptions) can provide more reliable inferences for Mendelian randomization investigations. A causal conclusion is more plausible in cases where multiple methods suggest a causal effect. We suggest that the IVW method using robust regression is a worthwhile method to apply in addition to the simple median and other methods, and that the use of penalized weights may be valuable in some situations.

Acknowledgements

Stephen Burgess is supported by the Wellcome Trust (grant number 100114). Jack Bowden is supported by a Methodology Research Fellowship from the Medical Research Council (grant number MR/N501906/1). Frank Dudbridge is supported by the Medical Research Council (grant number K006215). Simon G. Thompson is supported by the British Heart Foundation (grant number CH/12/2/29428).

References

[1] Greenland S, Robins J. Identifiability, exchangeability, and epidemiological confounding. *International Journal of Epidemiology* 1986; 15(3):413–419, doi: 10.1093/ije/15.3.413.

[2] Hernán M, Robins J. *Causal Inference*. Chapman & Hall/CRC Press, 2016. Available at http://www.hsph.harvard.edu/faculty/miguel-hernan/causal-inference-book/.

[3] Holland P. Statistics and causal inference. *Journal of the American Statistical Association* 1986; 81(396):945–960.
[4] Rubin D. Inference and missing data. *Biometrika* 1976; 63(3):581–592, doi:10.1093/biomet/63.3.581.

[5] Rubin D. Estimating causal effects of treatments in randomized and nonrandomized studies. *Journal of Educational Psychology* 1974; 66(5):688–701, doi:10.1037/h0037350.

[6] Greenland S. An introduction to instrumental variables for epidemiologists. *International Journal of Epidemiology* 2000; 29(4):722–729, doi:10.1093/ije/29.4.722.

[7] Clarke PS, Windmeijer F. Instrumental variable estimators for binary outcomes. *Journal of the American Statistical Association* 2012; 107(500):1638–1652, doi:10.1080/01621459.2012.734171.

[8] Small DS. Sensitivity analysis for instrumental variables regression with overidentifying restrictions. *Journal of the American Statistical Association* 2007; 102(479):1049–1058, doi:10.1198/016214507000000608.

[9] Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. *Statistical Methods in Medical Research* 2007; 16(4):309–330, doi:10.1177/0962280206077743.

[10] Davey Smith G, Ebrahim S. ‘Mendelian randomization’: can genetic epidemiology contribute to understanding environmental determinants of disease? *International Journal of Epidemiology* 2003; 32(1):1–22, doi:10.1093/ije/dyg070.

[11] Burgess S, Thompson SG. *Mendelian randomization: methods for using genetic variants in causal estimation*. Chapman & Hall, 2015.

[12] Lawlor D, Harbord R, Sterne J, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Statistics in Medicine* 2008; 27(8):1133–1163, doi:10.1002/sim.3034.

[13] Speliotes E, Willer C, Berndt S, Monda K, Thorleifsson G, Jackson A, Allen HL, Lindgren C, Luan J, Mägi R, et al.. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature Genetics* 2010; 42(11):937–948, doi:10.1038/ng.686.

[14] The International Consortium for Blood Pressure Genome-Wide Association Studies. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011; 478:103–109, doi:10.1038/nature10405.

[15] Burgess S, Scott R, Timpson N, Davey Smith G, Thompson S, EPIC-InterAct Consortium. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *European Journal of Epidemiology* 2015; 30(7):543–552, doi:10.1007/s10654-015-0011-z.

[16] Scott RA, Lagou V, Welch RP, Wheeler E, Montasser ME, Luan J, Mägi R, Strawbridge RJ, Rehnberg E, Gustafsson S, et al.. Large-scale association
analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nature Genetics* 2012; **44**(9):991–1005, doi: 10.1038/ng.2385.

[17] Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 2013; **381**:1371–1379, doi:10.1016/s0140-6736(12)62129-1.

[18] Burgess S, Butterworth A, Thompson S. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genetic Epidemiology* 2013; **37**(7):658–665, doi:10.1002/gepi.21758.

[19] Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *International Journal of Epidemiology* 2015; **44**(2):512–525.

[20] Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genetic Epidemiology* 2016; **40**(4):304–314, doi:10.1002/gepi.21965.

[21] Pearl J. *Causality: models, reasoning, and inference*. Cambridge University Press, 2000.

[22] Han C. Detecting invalid instruments using L1-GMM. *Economics Letters* 2008; **101**:285–287.

[23] Wald A. The fitting of straight lines if both variables are subject to error. *Annals of Mathematical Statistics* 1940; **11**(3):284–300.

[24] Thomas D, Lawlor D, Thompson J. Re: Estimation of bias in nongenetic observational studies using “Mendelian triangulation” by Bautista et al. *Annals of Epidemiology* 2007; **17**(7):511–513, doi:10.1016/j.annepidem.2006.12.005.

[25] Burgess S, Dudbridge F, Thompson S. Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods. *Statistics in Medicine* 2016; **35**(11):1880–1906, doi: 10.1002/sim.6835.

[26] Burgess S, Bowden J. Integrating summarized data from multiple genetic variants in Mendelian randomization: bias and coverage properties of inverse-variance weighted methods. *arXiv* 2015; **1512.04486**. Available at http://arxiv.org/abs/1512.04486.

[27] Kang H, Zhang A, Cai T, Small D. Instrumental variables estimation with some invalid instruments, and its application to Mendelian randomisation. *Journal of the American Statistical Association* 2015; doi:10.1080/01621459.2014.994705.

[28] Thompson S, Sharp S. Explaining heterogeneity in meta-analysis: a comparison of methods. *Statistics in Medicine* 1999; **18**(20):2693–2708.
[29] Huber PJ. *Robust statistics*. Springer, 2011.

[30] Koller M, Stahel WA. Sharpening wald-type inference in robust regression for small samples. *Computational Statistics & Data Analysis* 2011; 55(8):2504–2515, doi:10.1016/j.csda.2011.02.014.

[31] Yohai VJ. High breakdown-point and high efficiency robust estimates for regression. *The Annals of Statistics* 1987; 15(20):642–656, doi:10.1214/aos/1176350366.

[32] Mosteller F, Tukey JW. *Data analysis and regression: a second course in statistics*. Addison-Wesley Series in Behavioral Science: Quantitative Methods, 1977.

[33] Rousseeuw P, Croux C, Todorov V, Ruckstuhl A, Salibian-Barrera M, Verbeke T, Koller M, Maechler M. *robustbase: Basic Robust Statistics* 2015. URL [http://cran.r-project.org/package=robustbase](http://cran.r-project.org/package=robustbase), r package version 0.92-5.

[34] Rücker G, Schwarzer G, Carpenter JR, Binder H, Schumacher M. Treatment-effect estimates adjusted for small-study effects via a limit meta-analysis. *Biostatistics* 2011; 12(1):122–142.

[35] R Core Team. *R: A Language and Environment for Statistical Computing. Version 3.1.2 (Pumpkin Helmet)*. R Foundation for Statistical Computing, Vienna, Austria 2014. URL [http://www.R-project.org/](http://www.R-project.org/).

[36] Bowden J, Del Greco F, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for Mendelian randomization analyses using MR-Egger regression: the role of the $I^2$ statistic. Available from the authors.

[37] Burgess S, Thompson S. Bias in causal estimates from Mendelian randomization studies with weak instruments. *Statistics in Medicine* 2011; 30(11):1312–1323, doi:10.1002/sim.4197.

[38] Pierce B, Burgess S. Efficient design for Mendelian randomization studies: sub-sample and two-sample instrumental variable estimators. *American Journal of Epidemiology* 2013; 178(7):1177–1184, doi:10.1093/aje/kwt084.

[39] Frost C, Thompson S. Correcting for regression dilution bias: comparison of methods for a single predictor variable. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* 2000; 163(2):173–189, doi:10.1111/1467-985x.00164.

[40] Staiger D, Stock J. Instrumental variables regression with weak instruments. *Econometrica* 1997; 65(3):557–586.

[41] Cook JR, Stefanski LA. Simulation-extrapolation estimation in parametric measurement error models. *Journal of the American Statistical Association* 1994; 89(428):1314–1328, doi:10.1080/01621459.1994.10476871.
[42] Coodin S. Body mass index in persons with schizophrenia. *Canadian Journal of Psychiatry* 2001; 46(6):549–555.

[43] Allison DB, Fontaine KR, Heo M, Mentore JL, Cappelleri JC, Chandler LP, Cheskin LJ. The distribution of body mass index among individuals with and without schizophrenia. *The Journal of Clinical Psychiatry* 1999; 60(4):215–220, doi:10.4088/jcp.v60n0402.

[44] Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015; 518(7538):197–206, doi: 10.1038/nature14177.

[45] Burgess S, Bowden J, Fall T, Ingelsson E, Thompson S. Sensitivity analyses for robust causal inference from Mendelian randomization analyses with multiple genetic variants. *Epidemiology* 2016; Accepted manuscript; awaiting proofs.

[46] Swanson S, Hernán M. Commentary: how to report instrumental variable analyses (suggestions welcome). *Epidemiology* 2013; 24(3):370–374, doi:10.1097/ede.0b013e31828d0590.

[47] Imbens GW, Angrist JD. Identification and estimation of local average treatment effects. *Econometrica* 1994; 62(2):467–475, doi:10.2307/2951620.

[48] Robins JM. *Health service research methodology: a focus on AIDS*, chap. The analysis of randomized and nonrandomized AIDS treatment trials using a new approach to causal inference in longitudinal studies. National Center for Health Services Research, 1989; 113–159.

[49] Hernán M, Robins J. Instruments for causal inference: an epidemiologist’s dream? *Epidemiology* 2006; 17(4):360–372, doi:10.1097/01.ede.0000222409.00878.37.

[50] Burgess S, Butterworth AS, Thompson JR. Beyond Mendelian randomization: how to interpret evidence of shared genetic predictors. *Journal of Clinical Epidemiology* 2015; doi:10.1016/j.jclinepi.2015.08.001.

[51] Kolesár M, Chetty R, Friedman J, Glaser E, Imbens G. Identification and inference with many invalid instruments. *Journal of Business & Economic Statistics* 2014; doi:10.1080/07350015.2014.978175.

[52] Windmeijer F, Farbmacher H, Davies N, Davey Smith G, White I. Selecting (in)valid instruments for instrumental variables estimation 2015. Available at http://www.hec.unil.ch/documents/seminars/iems/1849.pdf.
### Table 1

| Method                              | Mean  | SD   | Mean SE | Power | NA   |
|-------------------------------------|-------|------|---------|-------|------|
| **Null causal effect: \( \theta = 0 \)** |       |      |         |       |      |
| Standard, no intercept              | 0.000 | 0.044| 0.047   | 3.9   | -    |
| Standard, intercept                 | 0.002 | 0.125| 0.133   | 3.8   | -    |
| Robust, no intercept                | 0.000 | 0.046| 0.050   | 4.5   | 1    |
| Robust, intercept                   | 0.002 | 0.130| 0.141   | 5.7   | 4    |
| Penalized standard, no intercept    | 0.000 | 0.046| 0.046   | 5.2   | -    |
| Penalized standard, intercept       | 0.002 | 0.130| 0.130   | 4.8   | -    |
| Penalized robust, no intercept      | 0.000 | 0.047| 0.047   | 5.9   | 2    |
| Penalized robust, intercept         | 0.001 | 0.132| 0.134   | 7.1   | 5    |
| Simple median                       | 0.000 | 0.059| 0.070   | 1.8   | -    |
| Weighted median                     | 0.001 | 0.056| 0.064   | 2.1   | -    |
| Penalized weighted median           | 0.001 | 0.059| 0.064   | 3.1   | -    |
| **Positive causal effect: \( \theta = +0.1 \)** |       |      |         |       |      |
| Standard, no intercept              | 0.096 | 0.047| 0.050   | 49.3  | -    |
| Standard, intercept                 | 0.065 | 0.136| 0.141   | 6.7   | -    |
| Robust, no intercept                | 0.096 | 0.048| 0.052   | 46.1  | 2    |
| Robust, intercept                   | 0.064 | 0.140| 0.148   | 8.7   | 4    |
| Penalized standard, no intercept    | 0.096 | 0.049| 0.048   | 51.8  | -    |
| Penalized standard, intercept       | 0.064 | 0.140| 0.137   | 8.3   | -    |
| Penalized robust, no intercept      | 0.096 | 0.050| 0.050   | 50.1  | 2    |
| Penalized robust, intercept         | 0.064 | 0.142| 0.140   | 10.3  | 5    |
| Simple median                       | 0.101 | 0.063| 0.074   | 24.7  | -    |
| Weighted median                     | 0.093 | 0.059| 0.067   | 25.7  | -    |
| Penalized weighted median           | 0.093 | 0.062| 0.067   | 26.5  | -    |

Table 1: Mean, standard deviation (SD), mean standard error (mean SE) of estimates, and empirical power (%) from weighted linear regression models (weights are penalized where indicated) using standard and robust regression, without and with an intercept term, and median-based methods for Scenario 1.

1Number of the 10,000 simulations that failed to report a standard error.
2This is the standard inverse-variance weighted (IVW) method.
3This is the MR-Egger method.
## Table 2: Mean, standard deviation (SD) of estimates, and empirical power (%) from weighted linear regression models (weights are not penalized) using standard and robust regression, without and with an intercept term, and simple and weighted median methods for Scenarios 2, 3, and 4. (Note: power with a null causal effect is the Type 1 error rate.)

| Method                | Scenario 2 |          | Scenario 3 |          | Scenario 4 |          |
|-----------------------|------------|----------|------------|----------|------------|----------|
|                       | Mean | SD   | Power | Mean | SD   | Power | Mean | SD   | Power |
| **Null causal effect: \( \theta = 0 \)** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| 0.000 | 0.069 | 5.4   | 0.067 | 0.065 | 13.0  | 0.059 | 0.076 | 19.0  |
| Standard, intercept   | 0.001 | 0.197 | 5.6   | 0.002 | 0.192 | 5.8   | 0.148 | 0.245 | 30.7  |
| Robust, no intercept  | 0.000 | 0.052 | 5.1   | 0.022 | 0.054 | 6.1   | 0.017 | 0.058 | 5.6   |
| Robust, intercept     | 0.001 | 0.153 | 6.8   | 0.001 | 0.154 | 6.5   | 0.081 | 0.205 | 11.5  |
| Simple median         | 0.000 | 0.066 | 2.8   | 0.030 | 0.066 | 4.5   | 0.013 | 0.066 | 3.2   |
| Weighted median       | 0.000 | 0.061 | 3.1   | 0.024 | 0.061 | 4.3   | 0.040 | 0.077 | 10.6  |
| **Proportion of invalid instrumental variables: 0.1** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| -0.001| 0.087 | 6.1   | 0.135 | 0.084 | 34.8  | 0.112 | 0.087 | 34.0  |
| Standard, intercept   | 0.003 | 0.251 | 6.2   | 0.008 | 0.234 | 6.1   | 0.255 | 0.260 | 43.9  |
| Robust, no intercept  | 0.000 | 0.064 | 5.4   | 0.062 | 0.074 | 10.4  | 0.049 | 0.080 | 9.7   |
| Robust, intercept     | 0.003 | 0.192 | 7.1   | 0.006 | 0.192 | 7.0   | 0.207 | 0.269 | 24.9  |
| Simple median         | -0.001| 0.074 | 3.8   | 0.067 | 0.078 | 11.3  | 0.027 | 0.074 | 4.9   |
| Weighted median       | 0.000 | 0.069 | 4.5   | 0.054 | 0.072 | 11.5  | 0.088 | 0.103 | 26.4  |
| **Proportion of invalid instrumental variables: 0.2** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| 0.001 | 0.103 | 5.9   | 0.204 | 0.093 | 59.3  | 0.161 | 0.092 | 48.9  |
| Standard, intercept   | 0.000 | 0.288 | 5.9   | 0.005 | 0.263 | 6.0   | 0.327 | 0.256 | 50.6  |
| Robust, no intercept  | 0.001 | 0.082 | 5.7   | 0.122 | 0.100 | 19.7  | 0.099 | 0.102 | 19.3  |
| Robust, intercept     | 0.003 | 0.243 | 6.3   | 0.005 | 0.239 | 7.5   | 0.332 | 0.293 | 42.0  |
| Simple median         | 0.000 | 0.082 | 5.1   | 0.115 | 0.094 | 25.0  | 0.046 | 0.084 | 8.6   |
| Weighted median       | 0.001 | 0.079 | 6.7   | 0.094 | 0.090 | 24.3  | 0.148 | 0.125 | 46.2  |
| **Proportion of invalid instrumental variables: 0.3** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| 0.095 | 0.070 | 32.6  | 0.162 | 0.069 | 69.2  | 0.155 | 0.078 | 63.3  |
| Standard, intercept   | 0.066 | 0.202 | 7.1   | 0.066 | 0.196 | 7.0   | 0.221 | 0.252 | 38.0  |
| Robust, no intercept  | 0.095 | 0.055 | 40.6  | 0.120 | 0.057 | 53.8  | 0.114 | 0.062 | 45.2  |
| Robust, intercept     | 0.066 | 0.162 | 9.0   | 0.066 | 0.162 | 8.8   | 0.149 | 0.218 | 15.8  |
| Simple median         | 0.100 | 0.070 | 23.4  | 0.132 | 0.070 | 38.5  | 0.114 | 0.070 | 29.4  |
| Weighted median       | 0.093 | 0.064 | 24.8  | 0.117 | 0.064 | 37.1  | 0.134 | 0.081 | 45.3  |
| **Positive causal effect: \( \theta = +0.1 \)** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| 0.095 | 0.089 | 22.6  | 0.230 | 0.085 | 84.0  | 0.208 | 0.089 | 73.2  |
| Standard, intercept   | 0.068 | 0.255 | 7.3   | 0.073 | 0.238 | 7.0   | 0.335 | 0.266 | 52.0  |
| Robust, no intercept  | 0.096 | 0.067 | 32.5  | 0.162 | 0.078 | 58.5  | 0.148 | 0.084 | 45.6  |
| Robust, intercept     | 0.069 | 0.201 | 9.1   | 0.071 | 0.201 | 8.8   | 0.278 | 0.281 | 29.5  |
| Simple median         | 0.100 | 0.077 | 22.9  | 0.170 | 0.083 | 53.3  | 0.129 | 0.078 | 33.9  |
| Weighted median       | 0.093 | 0.072 | 24.6  | 0.149 | 0.077 | 50.8  | 0.185 | 0.107 | 62.5  |
| **Proportion of invalid instrumental variables: 0.2** |        |        |        |        |        |        |        |        |        |
| Standard, no intercept| 0.097 | 0.105 | 18.6  | 0.299 | 0.095 | 93.8  | 0.257 | 0.094 | 81.6  |
| Standard, intercept   | 0.065 | 0.291 | 6.5   | 0.070 | 0.267 | 6.9   | 0.411 | 0.261 | 59.9  |
| Robust, no intercept  | 0.096 | 0.085 | 25.2  | 0.223 | 0.102 | 64.2  | 0.200 | 0.104 | 52.7  |
| Robust, intercept     | 0.068 | 0.251 | 7.7   | 0.070 | 0.247 | 8.8   | 0.406 | 0.304 | 46.7  |
| Simple median         | 0.101 | 0.087 | 22.5  | 0.221 | 0.100 | 69.2  | 0.148 | 0.089 | 38.9  |
| Weighted median       | 0.094 | 0.083 | 24.9  | 0.191 | 0.095 | 65.8  | 0.245 | 0.128 | 76.8  |
| Method                          | Scenario 2 |                  | Scenario 3 |                  | Scenario 4 |                  |
|--------------------------------|------------|-----------------|------------|-----------------|------------|-----------------|
|                                | Mean       | SD              | Mean       | SD              | Mean       | SD              |
| Null causal effect: $\theta = 0$ | 0.000      | 0.051           | 0.022      | 0.053           | 0.019      | 0.056           |
| Proportion of invalid          | 0.1        |                 | 0.1        |                 | 0.1        |                 |
| instrumental variables:        |            |                 |            |                 |            |                 |
| Penalized standard, no intercept | 0.000      | 0.052           | 0.018      | 0.053           | 0.015      | 0.055           |
| Penalized robust, no intercept | 0.001      | 0.149           | 0.001      | 0.154           | 0.093      | 0.198           |
| Penalized robust, intercept    | 0.001      | 0.151           | 0.001      | 0.153           | 0.077      | 0.191           |
| Penalized weighted median      | 0.001      | 0.063           | 0.011      | 0.063           | 0.016      | 0.074           |
| Positive causal effect: $\theta = +0.1$ | 0.001      | 0.069           | 0.106      | 0.088           | 0.091      | 0.092           |
| Proportion of invalid          | 0.2        |                 | 0.2        |                 | 0.2        |                 |
| instrumental variables:        |            |                 |            |                 |            |                 |
| Penalized standard, no intercept | 0.004      | 0.178           | 0.006      | 0.194           | 0.026      | 0.241           |
| Penalized standard, intercept  | 0.000      | 0.059           | 0.046      | 0.066           | 0.038      | 0.070           |
| Penalized robust, no intercept | 0.003      | 0.178           | 0.005      | 0.190           | 0.177      | 0.241           |
| Penalized robust, intercept    | 0.000      | 0.070           | 0.026      | 0.073           | 0.050      | 0.108           |
| Penalized weighted median      | 0.001      | 0.066           | 0.106      | 0.088           | 0.091      | 0.092           |
| Proportion of invalid          | 0.3        |                 | 0.3        |                 | 0.3        |                 |
| instrumental variables:        |            |                 |            |                 |            |                 |
| Penalized standard, no intercept | 0.095      | 0.054           | 0.219      | 0.067           | 0.110      | 0.079           |
| Penalized standard, intercept  | 0.065      | 0.158           | 0.066      | 0.162           | 0.164      | 0.209           |
| Penalized robust, no intercept | 0.095      | 0.055           | 0.115      | 0.056           | 0.111      | 0.059           |
| Penalized robust, intercept    | 0.065      | 0.159           | 0.066      | 0.161           | 0.147      | 0.202           |
| Penalized weighted median      | 0.093      | 0.066           | 0.104      | 0.067           | 0.110      | 0.079           |
| Positive causal effect: $\theta = +0.1$ | 0.095      | 0.062           | 0.154      | 0.071           | 0.147      | 0.077           |
| Proportion of invalid          | 0.2        |                 | 0.2        |                 | 0.2        |                 |
| instrumental variables:        |            |                 |            |                 |            |                 |
| Penalized standard, no intercept | 0.069      | 0.186           | 0.070      | 0.202           | 0.282      | 0.252           |
| Penalized standard, intercept  | 0.096      | 0.062           | 0.145      | 0.070           | 0.136      | 0.074           |
| Penalized robust, no intercept | 0.069      | 0.187           | 0.070      | 0.198           | 0.254      | 0.253           |
| Penalized robust, intercept    | 0.093      | 0.074           | 0.148      | 0.078           | 0.147      | 0.113           |
| Penalized weighted median      | 0.097      | 0.073           | 0.205      | 0.091           | 0.190      | 0.096           |
| Proportion of invalid          | 0.3        |                 | 0.3        |                 | 0.3        |                 |
| instrumental variables:        |            |                 |            |                 |            |                 |

Table 3: Mean, standard deviation (SD) of estimates, and empirical power (%) from weighted linear regression models (weights are penalized) using standard and robust regression, without and with an intercept term, and penalized weighted median method for Scenarios 2, 3, and 4.
Table 4: Proportion of simulated datasets for which the simple median and robust regression with no intercept (robust IVW) methods rejected the causal null (left), empirical power of the intercept test in MR-Egger method for detecting directional pleiotropy and/or violation of the InSIDE assumption in all scenarios.

| Proportion invalid | Simple median and robust IVW methods | MR-Egger intercept test |
|---------------------|--------------------------------------|-------------------------|
|                     | Scenario 1 2 3 4                     | Scenario 1 2 3 4         |
| Null causal effect: $\theta = 0$ |                                      |                         |
| 0%                  | 0.8%               -               -            | 3.8%       -     -     -             |
| 10%                 | -                  1.2%            2.2%          | 1.5%       -     5.5%   6.4%   24.4%   |
| 20%                 | -                  1.6%            5.5%          | 2.5%       -     6.0%   9.4%   31.2%   |
| 30%                 | -                  2.0%            14.1%         | 6.2%       -     5.9%   13.1%  32.8%   |
| Positive causal effect: $\theta = +0.1$ |                                      |                         |
| 0%                  | 21.2%               -               -            | 4.7%       -     -     -             |
| 10%                 | -                  19.1%           33.0%         | 23.6%      -     5.8%   8.0%   21.8%   |
| 20%                 | -                  16.7%           44.1%         | 26.6%      -     6.1%   11.1%  28.2%   |
| 30%                 | -                  14.6%           55.8%         | 31.8%      -     6.0%   15.3%  30.0%   |

Table 5: Estimates (standard errors, SE) and 95% confidence intervals (CI, calculated as estimate $\pm 1.96$ standard errors) of causal effect of body mass index on schizophrenia risk (log odds ratio for schizophrenia per 1 standard deviation increase in body mass index).

| Method                  | Non-penalized weights | Penalized weights |
|-------------------------|-----------------------|-------------------|
|                         | Estimate (SE) 95% CI   | Estimate (SE) 95% CI |
| Standard, no intercept  | -0.031 (0.100) -0.227, 0.165 | -0.034 (0.057) -0.147, 0.078 |
| Standard, intercept     | 0.336 (0.241) -0.136, 0.808 | 0.154 (0.143) -0.127, 0.435 |
| Robust, no intercept    | -0.024 (0.079) -0.180, 0.132 | -0.033 (0.062) -0.154, 0.089 |
| Robust, intercept       | 0.255 (0.212) -0.162, 0.671 | 0.142 (0.150) -0.152, 0.436 |
| Simple median           | -0.073 (0.088) -0.244, 0.098 | -     -     -     -             |
| Weighted median         | -0.075 (0.087) -0.246, 0.096 | -0.076 (0.090) -0.253, 0.100 |
| Method                                      | Description                                                                 |
|---------------------------------------------|-----------------------------------------------------------------------------|
| Inverse-variance weighted (IVW) method      | Standard regression with inverse-variance weights and intercept term set to zero. |
| MR-Egger method                             | Standard regression with inverse-variance weights and intercept term estimated. |
| Median-based method                         | Simple median method is the median of the causal estimates based on the individual candidate instruments. Weighted median method uses inverse-variance weights so that more precise estimates receive more weight in the analysis. |
| Robust regression (MM-estimation with bisquare objective function) | Standard regression in either the IVW (no intercept) or the MR-Egger (intercept) method can be replaced with robust regression. |
| Penalization of weights                     | Inverse-variance weights in either the IVW, MR-Egger, or weighted median method can be replaced with weights that depend on the heterogeneity of the causal estimates – candidate instruments with outlying estimates are downweighted depending on the degree of heterogeneity. |

Table 6: Summary of methods investigated in this paper.
Figures

Figure 1: Directed acyclic graph of graphical instrumental variable assumptions.

Figure 2: Decomposition of association with the outcome $Y$ for genetic variant $G_j$ into indirect (causal) effect via the exposure $X$ and direct (pleiotropic) effect (see equation 6).
Figure 3: Estimated genetic associations and 95% confidence intervals with body mass index (BMI, standard deviation units) and with schizophrenia risk (log odds ratios) for 97 genetic variants.
Appendix

A1 Software code

We provide R code to implement the methods discussed in this paper. The associations of the candidate instruments with the exposure are denoted $\beta_{XG}$ with standard errors $se_{\beta_{XG}}$. The associations of the candidate instruments with the outcome are denoted $\beta_{YG}$ with standard errors $se_{\beta_{YG}}$. We assume that the candidate instruments are uncorrelated in their distributions, as is common in applied Mendelian randomization investigations.

Inverse-variance weighted estimate:

The inverse-variance weighted (IVW) estimate can be calculated by weighted linear regression:

```
betaIVW = summary(lm(betaYG~betaXG-1, weights=sebetaYG ^-2))$coef[1]
sebetaIVW.fixed = summary(lm(betaYG~betaXG-1, weights= sebetaYG^-2))$coef[1,2]/
summary(lm(betaYG~betaXG-1, weights=sebetaYG^-2))$sigma
sebetaIVW.random = summary(lm(betaYG~betaXG-1, weights =sebetaYG^-2))$coef[1,2]/
min(summary(lm(betaYG~betaXG-1, weights=sebetaYG^-2))$sigma,1)
```

In the fixed-effect model, we divide the reported standard error by the estimated residual standard error, to fix the residual standard error to take the value 1 \[28\]. In the multiplicative random-effects model, we divide by the estimated residual standard error in the case of underdispersion (the variability in the genetic associations is less than would be expected by chance alone). But in the case of overdispersion (that is, heterogeneity of causal effect estimates), no correction is made. The point estimate is unaffected by the choice of a fixed- or multiplicative random-effects model.

Alternatively, the inverse-variance weighted estimate can be calculated by meta-analysis, or via a simple formula:

```
library(meta)
betaIVW = metagen(betaYG/betaXG, abs(sebetaYG/betaXG))$TE.fixed
sebetaIVW.fixed = metagen(betaYG/betaXG, abs(sebetaYG/betaXG))$seTE.fixed
# simple formula
betaIVW = sum(betaYG*betaXG*sebetaYG^-2)/sum(betaXG^2*sebetaYG^-2)
sebetaIVW.fixed = 1/sqrt(sum(betaXG^2*sebetaYG^-2))
```

The meta-analysis method can be used to perform an additive random-effects analysis, which makes a different parametric assumption about the heterogeneity between causal estimates compared with the multiplicative random-effect analysis \[26\]. While the causal estimates from the fixed-effect and multiplicative random-effects analyses are the same, the estimate from the additive random-effects analysis differs.
MR-Egger regression:

The MR-Egger method is equivalent to the IVW method calculated using weighted regression, except that the intercept term is estimated rather than being set to zero. A test as to whether the intercept term is equal to zero is a test of directional pleiotropy. A random-effects model should be used for inference as a fixed-effect model is not justifiable when the candidate instruments are not all valid.

```r
# coding of genetic variants
betaYG = betaYG*sign(betaXG); betaXG = abs(betaXG)
# causal estimate
betaEgger = summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$coef[2,1]
sebetaEgger.random = summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$coef[2,2]/
                     min(summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$sigma, 1)
betaEgger.lower = betaEgger-qt(0.975,df=length(betaXG)-2)*sebetaEgger.random
betaEgger.upper = betaEgger+qt(0.975,df=length(betaXG)-2)*sebetaEgger.random
p.causal.random = 2*(1-pt(abs(betaEgger/sebetaEgger.random),df=length(betaXG)-2))
# test for directional pleiotropy
interEgger = summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$coef[1,1]
seinterEgger.random = summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$coef[1,2]/
                      min(summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$sigma, 1)
p.dpleio.random = 2*(1-pt(abs(interEgger/seinterEgger.random),df=length(betaXG)-2))

In this code, we use a t-distribution with \( J - 2 \) degrees of freedom for inference. If there is underdispersion, then the t-distribution may be overly conservative, as the t-distribution assumes that the residual standard error is estimated (in case of underdispersion, the residual standard error is set to 1). Hence, if the residual standard error is less than one, either a confidence interval using a residual standard error of 1 and a z-distribution, or else a confidence interval using the estimated residual standard error and a t-distribution may be preferred (the wider of these two intervals should be preferred – both of these will be narrower than the above confidence interval).

```r
sigmaEgger = summary(lm(betaYG~betaXG, weights=sebetaYG^-2))$sigma
betaEgger.lower = ifelse(sigmaEgger<1, min(betaEgger-qnorm(0.975)*sebetaEgger.random, 
                              betaEgger-qt(0.975,df=length(betaXG)-2)*sebetaEgger.random)*sigmaEgger, 
                              betaEgger-qt(0.975,df=length(betaXG)-2)*sebetaEgger.random)
betaEgger.upper = ifelse(sigmaEgger<1, max(betaEgger+qnorm(0.975)*sebetaEgger.random, 
                               betaEgger+qt(0.975,df=length(betaXG)-2)*sebetaEgger.random)*sigmaEgger, 
                               betaEgger+qt(0.975,df=length(betaXG)-2)*sebetaEgger.random)
```

Median-based method:

The median-based method calculates the median (or weighted median) of the causal estimates from each candidate instrument. This code calculates the simple median, weighted median, and penalized weighted median, employing bootstrapping to obtain a standard error that can used to provide a confidence interval.

```r
weighted.median <- function(betaIV.in, weights.in) {
  betaIV.order = betaIV.in[order(betaIV.in)]
  weights.order = weights.in[order(betaIV.in)]
  weights.sum = cumsum(weights.order)-0.5*weights.order
  weights.sum = weights.sum/sum(weights.order)
  below = max(which(weights.sum<0.5))
  weighted.est = betaIV.order[below] + (betaIV.order[below+1]-betaIV.order[below])*
                  (0.5-weights.sum[below])/(weights.sum[below+1]-weights.sum[below])
```

32
weighted.median.boot = function(betaXG.in, betaYG.in, sebetaXG.in, sebetaYG.in, weights.in){
  # the standard error is estimated based on 1000 bootstrap samples
  med = NULL
  for(i in 1:1000){
    betaXG.boot = rnorm(length(betaXG.in), mean=betaXG.in, sd=sebetaXG.in)
    betaYG.boot = rnorm(length(betaYG.in), mean=betaYG.in, sd=sebetaYG.in)
    betaIV.boot = betaYG.boot/betaXG.boot
    med[i] = weighted.median(betaIV.boot, weights.in)
  }
  return(sd(med))}

betaIV = betaYG/betaXG
weights = rep(1, length(betaXG)) # unweighted median
betaSIMPLEMED = weighted.median(betaIV, weights)
sebetaSIMPLEMED = weighted.median.boot(betaXG, betaYG, sebetaXG, sebetaYG, weights)
lowerSIMPLEMED = betaSIMPLEMED-qnorm(0.975)*sebetaSIMPLEMED
upperSIMPLEMED = betaSIMPLEMED+qnorm(0.975)*sebetaSIMPLEMED

weights = (sebetaYG/betaXG)^-2 # weighted median using inverse-variance weights
betaWEIGHTEDMED = weighted.median(betaIV, weights)
sebetaWEIGHTEDMED = weighted.median.boot(betaXG, betaYG, sebetaXG, sebetaYG, weights)
lowerWEIGHTEDMED = betaWEIGHTEDMED-qnorm(0.975)*sebetaWEIGHTEDMED
upperWEIGHTEDMED = betaWEIGHTEDMED+qnorm(0.975)*sebetaWEIGHTEDMED

# penalized weighted median
penalty = pchisq(weights*(betaIV-betaWEIGHTEDMED)^2, df=1, lower.tail=FALSE)
pen.weights = (sebetaYG/betaXG)^-2*pmin(1, penalty*20) # penalized weights
betaPENALIZEDMED = weighted.median(betaIV, pen.weights)
sebetaPENALIZEDMED = weighted.median.boot(betaXG, betaYG, sebetaXG, sebetaYG, pen.weights)
lowerPENALIZEDMED = betaPENALIZEDMED-qnorm(0.975)*sebetaPENALIZEDMED
upperPENALIZEDMED = betaPENALIZEDMED+qnorm(0.975)*sebetaPENALIZEDMED

Robust regression:
The IVW and MR-Egger methods can be performed using robust regression (in particular, MM-estimation using Tukey’s bisquare objective function) rather than standard linear regression:

```r
library(robustbase)
betaIVW.robust = summary(lmrob(betaYG~betaXG-1, weights=sebetaYG^-2, k.max=500))$coef[1]
sebetaIVW.robust.fixed = summary(lmrob(betaYG~betaXG-1, weights=sebetaYG^-2, k.max=500))$coef[1,2]/summary(lmrob(betaYG~betaXG-1, weights=sebetaYG^-2, k.max=500))$sigma
sebetaIVW.robust.random = summary(lmrob(betaYG~betaXG-1, weights=sebetaYG^-2, k.max=500))$coef[2,2]/min(summary(lmrob(betaYG~betaXG-1, weights=sebetaYG^-2, k.max=500))$sigma,1)

betaEGGER.robust = summary(lmrob(betaYG~betaXG, weights=sebetaYG^-2, k.max=500))$coef[2]
sebetaEGGER.robust.random = summary(lmrob(betaYG~betaXG, weights=sebetaYG^-2, k.max=500))$coef[2,2]/min(summary(lmrob(betaYG~betaXG, weights=sebetaYG^-2, k.max=500))$sigma,1)
```

The `k.max` option sets the maximum number of steps evaluated to find initial parameter values in the S-step of the algorithm.

Penalized weights:
The IVW and MR-Egger methods can be performed using penalized weights:

```r
betaIVW = sum(betaYG*betaXG*sebetaYG^-2)/sum(betaXG^2*sebetaYG^-2)
pweights = pchisq(betaXG^2/sebetaYG^2*(betaYG/betaXG-betaIVW)^2, df=1, lower.tail=FALSE)
```
Penalized weights can also be used in conjunction with robust regression.
A2 Supplementary tables for simulation study

A.1 Number of simulations that failed to report a standard error

The numbers of simulations for the robust methods that failed to report a standard error in Scenarios 2 to 4 are provided in Web Table A1. The proportion of simulations was usually less than 1%, and was less than 2.5% in all cases.

| Method                        | Null causal effect | Positive causal effect |
|-------------------------------|--------------------|------------------------|
| Proportion invalid: 10% 20% 30% | 10% 20% 30%        |                        |
| Scenario 2: balanced pleiotropy, InSIDE satisfied |                    |                        |
| Robust, no intercept         | 1 2 0              | 0 1 0                  |
| Robust, intercept            | 5 20 24            | 12 18 24               |
| Penalized robust, no intercept | 4 5 18            | 0 10 12                |
| Penalized robust, intercept  | 15 42 97           | 10 23 80               |
| Scenario 3: directional pleiotropy, InSIDE satisfied |                    |                        |
| Robust, no intercept         | 1 0 0              | 0 1 1                  |
| Robust, intercept            | 2 18 15            | 5 10 11                |
| Penalized robust, no intercept | 4 5 8             | 1 2 3                  |
| Penalized robust, intercept  | 2 30 44            | 15 19 31               |
| Scenario 4: directional pleiotropy, InSIDE not satisfied |                    |                        |
| Robust, no intercept         | 2 15 34            | 3 15 26                |
| Robust, intercept            | 131 245 244        | 147 233 227            |
| Penalized robust, no intercept | 2 12 44           | 2 9 41                 |
| Penalized robust, intercept  | 24 69 102          | 31 51 92               |

Web Table A1: Number of the 10000 simulations that failed to report a standard error using the robust regression method in each of the simulation settings.

A.2 One-sample setting

The simulation study from the main body of the paper was repeated, except in a one-sample setting in which associations of the candidate instruments with the exposure and with the outcome were obtained in the same sample of 20000 individuals for the methods using non-penalized weights. Results are displayed in Web Table A2 (Scenario 1) and Web Table A3 (Scenarios 2 to 4).

A.3 Fewer candidate instruments

The simulation was also repeated in a two-sample setting with only 10 candidate instruments, to observe whether the robust methods were able to operate well with fewer instruments to detect violations of the instrumental variables assumptions. Results for Scenarios 2 to 4 are presented in Web Table A4.
Web Table A2: Mean, standard deviation (SD), mean standard error (mean SE) of estimates, and empirical power (%) from weighted linear regression models (weights are penalized where indicated) using standard and robust regression, without and with an intercept term, and median-based methods for Scenario 1 in one-sample setting (associations with exposure and with outcome are estimated in the same individuals).

| Method                                      | Scenario 1 |          |          | Power | NA |
|---------------------------------------------|------------|----------|----------|-------|----|
| Null causal effect: $\theta = 0$            |            | Mean     | SD       | Mean SE |     |
| Standard, no intercept                      | 0.024      | 0.044    | 0.047    | 6.8    | -  |
| Standard, intercept                         | 0.173      | 0.123    | 0.131    | 27.2   | -  |
| Robust, no intercept                        | 0.023      | 0.044    | 0.049    | 7.8    | 0  |
| Robust, intercept                           | 0.174      | 0.126    | 0.136    | 29.0   | 4  |
| Penalized standard, no intercept            | 0.024      | 0.045    | 0.046    | 8.5    | -  |
| Penalized standard, intercept               | 0.174      | 0.126    | 0.129    | 28.5   | -  |
| Penalized robust, no intercept              | 0.024      | 0.046    | 0.047    | 9.4    | 0  |
| Penalized robust, intercept                 | 0.174      | 0.127    | 0.131    | 31.0   | 3  |
| Simple median                               | 0.000      | 0.060    | 0.070    | 2.0    | -  |
| Weighted median                             | 0.038      | 0.054    | 0.063    | 5.5    | -  |
| Penalized weighted median                   | 0.038      | 0.057    | 0.063    | 6.5    | -  |
| Positive causal effect: $\theta = +0.1$     |            | Mean     | SD       | Mean SE |     |
| Standard, no intercept                      | 0.123      | 0.044    | 0.048    | 73.6   | -  |
| Standard, intercept                         | 0.271      | 0.121    | 0.136    | 53.1   | -  |
| Robust, no intercept                        | 0.122      | 0.045    | 0.050    | 69.3   | 0  |
| Robust, intercept                           | 0.271      | 0.125    | 0.143    | 52.4   | 3  |
| Penalized standard, no intercept            | 0.122      | 0.045    | 0.048    | 74.1   | -  |
| Penalized standard, intercept               | 0.271      | 0.123    | 0.135    | 53.8   | -  |
| Penalized robust, no intercept              | 0.122      | 0.046    | 0.049    | 71.6   | 1  |
| Penalized robust, intercept                 | 0.271      | 0.126    | 0.139    | 53.9   | 2  |
| Simple median                               | 0.099      | 0.059    | 0.073    | 25.4   | -  |
| Weighted median                             | 0.136      | 0.054    | 0.067    | 54.6   | -  |
| Penalized weighted median                   | 0.136      | 0.057    | 0.067    | 54.5   | -  |

1 Number of the 10 000 simulations that failed to report a standard error.
2 This is the standard inverse-variance weighted (IVW) method.
3 This is the MR-Egger method.
### Table A3

| Method                           | Scenario 2 |                     | Scenario 3 |                     | Scenario 4 |                     |
|----------------------------------|------------|---------------------|------------|---------------------|------------|---------------------|
|                                  | Mean       | SD                  | Power      | Mean                | SD         | Power               |
| Null causal effect: $\theta = 0$ | 0.023      | 0.069               | 7.3        | 0.090               | 0.066      | 24.5                |
| Standard, no intercept           | 0.174      | 0.197               | 19.4       | 0.175               | 0.190      | 19.2                |
| Robust, no intercept             | 0.023      | 0.052               | 8.3        | 0.045               | 0.053      | 13.2                |
| Robust, intercept                | 0.174      | 0.148               | 26.9       | 0.175               | 0.148      | 27.1                |
| Simple median                    | -0.001     | 0.066               | 2.9        | 0.028               | 0.064      | 4.5                 |
| Weighted median                  | 0.037      | 0.061               | 6.8        | 0.061               | 0.060      | 12.6                |
| Proportion of invalid IVs: 0.1   | 0.123      | 0.069               | 49.4       | 0.226               | 0.091      | 74.7                |
| Standard, no intercept           | 0.172      | 0.197               | 35.7       | 0.275               | 0.190      | 36.4                |
| Robust, no intercept             | 0.022      | 0.052               | 62.8       | 0.143               | 0.096      | 30.3                |
| Robust, intercept                | 0.172      | 0.234               | 49.2       | 0.176               | 0.232      | 17.6                |
| Simple median                    | -0.001     | 0.073               | 7.8        | 0.089               | 0.069      | 22.5                |
| Weighted median                  | 0.037      | 0.067               | 7.8        | 0.089               | 0.069      | 22.5                |
| Proportion of invalid IVs: 0.2   | 0.123      | 0.087               | 68.0       | 0.258               | 0.082      | 95.2                |
| Standard, no intercept           | 0.171      | 0.286               | 11.0       | 0.178               | 0.259      | 12.8                |
| Robust, no intercept             | 0.022      | 0.080               | 67.0       | 0.143               | 0.096      | 30.3                |
| Robust, intercept                | 0.172      | 0.234               | 17.5       | 0.176               | 0.232      | 17.6                |
| Simple median                    | -0.001     | 0.082               | 4.7        | 0.109               | 0.085      | 24.7                |
| Weighted median                  | 0.036      | 0.077               | 9.2        | 0.127               | 0.084      | 38.4                |
| Proportion of invalid IVs: 0.3   | 0.123      | 0.102               | 49.4       | 0.226               | 0.091      | 74.7                |
| Standard, no intercept           | 0.171      | 0.286               | 11.0       | 0.178               | 0.259      | 12.8                |
| Robust, no intercept             | 0.022      | 0.080               | 67.0       | 0.143               | 0.096      | 30.3                |
| Robust, intercept                | 0.172      | 0.234               | 17.5       | 0.176               | 0.232      | 17.6                |
| Simple median                    | -0.001     | 0.082               | 4.7        | 0.109               | 0.085      | 24.7                |
| Weighted median                  | 0.036      | 0.077               | 9.2        | 0.127               | 0.084      | 38.4                |

Positive causal effect: $\theta = +0.1$

| Method                           | Scenario 2 |                     | Scenario 3 |                     | Scenario 4 |                     |
|----------------------------------|------------|---------------------|------------|---------------------|------------|---------------------|
|                                  | Mean       | SD                  | Power      | Mean                | SD         | Power               |
| Standard, no intercept           | 0.123      | 0.069               | 49.4       | 0.190               | 0.066      | 87.2                |
| Standard, intercept              | 0.274      | 0.197               | 35.7       | 0.275               | 0.190      | 36.4                |
| Robust, no intercept             | 0.123      | 0.052               | 62.8       | 0.145               | 0.053      | 74.8                |
| Robust, intercept                | 0.274      | 0.148               | 49.2       | 0.275               | 0.148      | 49.2                |
| Simple median                    | 0.099      | 0.066               | 24.6       | 0.128               | 0.064      | 38.9                |
| Weighted median                  | 0.137      | 0.061               | 52.0       | 0.161               | 0.060      | 66.0                |
| Proportion of invalid IVs: 0.1   | 0.123      | 0.087               | 34.9       | 0.258               | 0.082      | 95.2                |
| Standard, no intercept           | 0.272      | 0.248               | 25.2       | 0.278               | 0.232      | 27.2                |
| Standard, intercept              | 0.123      | 0.063               | 52.4       | 0.184               | 0.072      | 76.3                |
| Robust, intercept                | 0.273      | 0.187               | 40.7       | 0.275               | 0.186      | 40.5                |
| Simple median                    | 0.099      | 0.073               | 23.9       | 0.164               | 0.073      | 54.6                |
| Weighted median                  | 0.137      | 0.067               | 49.8       | 0.189               | 0.069      | 76.4                |
| Proportion of invalid IVs: 0.2   | 0.123      | 0.087               | 34.9       | 0.258               | 0.082      | 95.2                |
| Standard, no intercept           | 0.272      | 0.248               | 25.2       | 0.278               | 0.232      | 27.2                |
| Standard, intercept              | 0.123      | 0.063               | 52.4       | 0.184               | 0.072      | 76.3                |
| Robust, intercept                | 0.273      | 0.187               | 40.7       | 0.275               | 0.186      | 40.5                |
| Simple median                    | 0.099      | 0.073               | 23.9       | 0.164               | 0.073      | 54.6                |
| Weighted median                  | 0.137      | 0.067               | 49.8       | 0.189               | 0.069      | 76.4                |
| Proportion of invalid IVs: 0.3   | 0.124      | 0.102               | 26.8       | 0.326               | 0.091      | 98.1                |
| Standard, no intercept           | 0.271      | 0.286               | 19.0       | 0.278               | 0.259      | 21.4                |
| Standard, intercept              | 0.122      | 0.080               | 40.2       | 0.243               | 0.096      | 76.8                |
| Robust, intercept                | 0.272      | 0.234               | 30.6       | 0.276               | 0.232      | 31.0                |
| Simple median                    | 0.099      | 0.082               | 23.2       | 0.209               | 0.085      | 69.5                |
| Weighted median                  | 0.136      | 0.077               | 46.6       | 0.227               | 0.084      | 84.6                |

Web Table A3: Mean, standard deviation (SD), mean standard error (mean SE) of estimates, and empirical power (%) from weighted linear regression models (weights are not penalized) using standard and robust regression, without and with an intercept term, and simple and weighted median methods for Scenarios 2, 3, and 4 in one-sample setting (associations with exposure and with outcome are estimated in the same individuals).
### Scenario 2

| Method                        | Mean  | SD   | Power |
|-------------------------------|-------|------|-------|
| Null causal effect: $\theta = 0$ |       |      |       |
| Standard, no intercept        | -0.001| 0.113| 5.5   |
| Standard, intercept           | -0.003| 0.353| 6.2   |
| Robust, no intercept          | 0.000 | 0.090| 7.0   |
| Robust, intercept             | 0.001 | 0.321| 12.5  |
| Simple median                 | -0.001| 0.105| 3.2   |
| Weighted median               | 0.000 | 0.098| 4.2   |

### Scenario 3

| Method                        | Mean  | SD   | Power |
|-------------------------------|-------|------|-------|
| Positive causal effect: $\theta = +0.1$ |       |      |       |
| Standard, no intercept        | 0.001 | 0.139| 5.8   |
| Standard, intercept           | 0.001 | 0.432| 7.1   |
| Robust, no intercept          | 0.002 | 0.111| 7.2   |
| Robust, intercept             | 0.002 | 0.398| 14.3  |
| Simple median                 | 0.000 | 0.117| 4.0   |
| Weighted median               | 0.001 | 0.113| 5.8   |

### Scenario 4

| Method                        | Mean  | SD   | Power |
|-------------------------------|-------|------|-------|
| Positive causal effect: $\theta = +0.1$ |       |      |       |
| Standard, no intercept        | 0.095 | 0.116| 18.1  |
| Standard, intercept           | 0.063 | 0.361| 6.8   |
| Robust, no intercept          | 0.096 | 0.095| 20.8  |
| Robust, intercept             | 0.066 | 0.337| 13.3  |
| Simple median                 | 0.101 | 0.111| 11.8  |
| Weighted median               | 0.093 | 0.104| 13.0  |

Web Table A4: Mean, standard deviation (SD), mean standard error (mean SE) of estimates, and empirical power (%) from weighted linear regression models (weights are not penalized) using standard and robust regression, without and with an intercept term, and simple and weighted median methods for Scenarios 2, 3, and 4 in two-sample setting with only 10 candidate instruments (25 candidate instruments are used in all other simulations).