Causal Effect Estimation in Mendelian Randomisation Studies - Evaluating a Novel Bayesian Approach To Genetic Pleiotropy Versus Established Weighted Median Methodology

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Contents

0.1 Acknowledgements

I would like to acknowledge

0.2 Contributions

Mine others

0.3 Statement of originality

I confirm that all work is my own except where indicated, that all sources are clearly referenced….

0.4 Word Count

Word count: 922

1 Introduction and Background

Mendelian randomisation (MR) is a methodology intended to support causal inference from observational data. It applies the principles of instrumental variable (IV) analysis to genetic data: naturally occurring genetic variants - "instruments" – with a known association to an exposure of interest are chosen, and by comparing the association of those same genetic instruments to an outcome of interest, genetic data can be used to investigate causal links between exposures and outcomes[1]. In theory, provided that the assumptions of IV analysis are met, random assignment of genetic variants from parents to offspring during meiosis can create a form of natural experiment, analogous to randomisation during a clinical trial – both measured and unmeasured confounders should be distributed evenly between the groups created, allowing valid causal inference after other sources of bias and random variation are accounted for[2].

Three key assumptions of IV analysis must be met [@ ***REF***]: 1. Relevance – the genetic variant must be associated with the exposure of interest 2. Independence – the genetic variant is independent of confounders of the relationship between exposure and outcome 3. Exclusion restriction – the genetic variant must not be associated with the outcome except via the exposure

If these assumptions are satisfied, the "causal effect estimate" of the exposure on the outcome can be estimated by the Wald ratio, i.e. by dividing the co-efficient of gene-outcome association by the co-efficient of gene-exposure, giving a numerical measure of strength of causal exposure-outcome association[3].

Figure X. Taken from Burgess et al 2016 (DAG)[4]

In practice, only the relevance assumption can be directly tested and proven, as independence and exclusion restriction depend on all possible confounders of the exposure-outcome association, both measured and unmeasured[@ ***REF***]. Threats to the independence assumption will vary depending on the population, exposure and outcome being studied[@ ***REF***]. Exclusion restriction is a particularly universal issue in MR, due to so-called (horizontal) genetic pleiotropy, where a single genetic variant may have multiple "pleiotropic" effects – i.e. it may influence several traits simultaneously. Such pleiotropic effects may be unknown and open unmeasured causal pathways between a genetic instrument and the outcome, separate to the path involving the exposure of interest, thus potentially biasing MR estimates of the association between exposure and outcome[5].

Although not possible to prove exclusion restriction for any MR study, several methods attempt to produce exposure-outcome causal effect estimates which are robust to violations of this assumption. A common approach is the Weighted Median Estimator (WME) method, proposed by Bowden et al[6].

In WME analysis, several genetic instruments are used to estimate the exposure-outcome causal effect; each instrument is known to be associated with the exposure of interest, but a proportion of these instruments may be invalid due to unknown pleiotropic genetic effects. Any genetic instrument causally linked an outcome via multiple pleiotropic causal pathways would be expected to exhibit a less consistent gene-outcome association than if only a single pathway mediated the gene-outcome relationship; this would be reflected in larger variance in causal effect estimates derived from invalid/pleiotropic genetic instruments. WME therefore assigns a weight to each genetic instrument's estimate of the causal effect according to the inverse of the variance of the estimate; these weighted effect estimates are used to construct a cumulative distribution function for probability of true causal effect size across the range of estimated values.

Causal effect estimates from each instrument are ordered by size, then used to create a cumulative distribution function for probability of true causal effect size. The relative contribution of each instrument's effect estimate to the probability distribution is weighted according to the inverse of the variance of the estimate. Genetic instruments whose causal effect estimates exhibit a large variance, which would be expected would therefore contribute less to….

In WME analysis, several genetic instruments are used to estimate the exposure-outcome causal effect. Any instrument linked to an outcome via multiple pleiotropic causal pathways will exhibit a less consistent gene-outcome association than a relationship mediated by a single pathway; this results in larger variance in causal estimates derived from invalid/pleiotropic genetic instruments. WME therefore assigns a weight to each genetic instrument's causal estimate according to the inverse of its variance, then constructs a cumulative

distribution function for probability of true causal effect size across the range of estimated values, before taking the 50th percentile of this distribution as a "weighted median estimate" of the true causal effect, theoretically producing consistent causal estimates even if up to 50% of the included information comes from invalid instruments.

2 References

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5. Hemani G, Bowden J, Smith GD. Evaluating the potential role of pleiotropy in Mendelian randomization studies. Human Molecular Genetics [Internet]. 2018 May [cited 2024 Oct 23];27(R2):R195. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6061876/

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A Appendix A: List of Abbreviations

B Appendix B: Simulation Code

```
# Load packages
 library(tidyverse)
 library(TwoSampleMR)
 library(rstan)
 library(here)
 library(cowplot)

# Load University of Edinburgh colour palette
 source(here("edin_uni_colours.R"))

# Load pre-formatted plot template - call to ggplot with UoE colours
 source(here("edin_fig_style.R"))

# Compile model for MR-Hevo
 mr.stanmodel <- stan_model(file= here("MRHevo_summarystats.stan"),
                                    model_name="MRHevo.summarystats", verbose=FALSE)
```

B.0.1 Generating Data and Models

The data generating model used was from Appendix 3 of Bowden et al (ref); the relevant section describing their model is reproduced below:

"...

$$U_i = \sum_{j=1}^{J} \phi_j G_{ij} + \epsilon_i^U \tag{1}$$

$$X_i = \sum_{j=1}^{J} \gamma_j G_{ij} + U_i + \epsilon_i^X \tag{2}$$

$$Y_i = \sum_{j=1}^{J} \alpha_j G_{ij} + \beta X_i + U_i + \epsilon_i^Y \tag{3}$$

for participants indexed by $i = 1, ..., N$, *and genetic instruments indexed by* $j = 1, ..., J$.

The error terms $\epsilon_i^U, \epsilon_i^X$ *and* ϵ_i^Y *were each drawn independently from standard normal distributions. The genetic effects on the xposure* j *are drawn from a uniform distribution between 0.03 and 0.1. Pleiotropic effects* α_j *and* ϕ_j *were set to zero if the genetic instrument was a alid instrumental variable. Otherwise (with probability 0.1, 0.2, or 0.3):*

1. In Scenario 1 (balanced pleiotropy, InSIDE satisfied), the α_j *parameter was drawn from a uniform distribution between* -0.2 *and 0.2.*

2. In Scenario 2 (directional pleiotropy, InSIDE satisfied), the α_j *parameter was drawn from a uniform distribution between 0 and 0.2.*

3. In Scenario 3 (directional pleiotropy, InSIDE not satisfied), the ϕ_j *parameter was drawn from a uniform distribution between* -0.2 *and 0.2.*

The causal effect of the exposure on the outcome was either $\beta X = 0$ *(null causal effect) or* $\beta X = 0.1$ *(positive causal effect). A total of 10 000 simulated datasets were generated for sample sizes of* $N = 10\ 000$ *and*

20 [sic] participants. Only the summary data, that is genetic associations with the exposure and with the outcome and their standard errors as estimated by univariate regression on the genetic instruments in turn, were used by the analysis methods. In the two-sample setting, data were generated on 2N participants, and genetic associations with the exposure were estimated in the first N participants, and genetic associations with the outcome in the second N participants."[6]

To reproduce this model, code was written in R to generate the relevant participant level data. First, a function (`simulate_MR_data`) was written which included arameters specified by Bowden et al, and also to allow testing of data simulation:

```
# Define function to create data generating model
# Arguments/default values based on Bowden et al
 simulate_MR_data <- function(n_participants = as.integer(),
                              n_instruments = as.integer(),
                              n_datasets = as.integer(),
                              prop_invalid = 0.1,
                              causal_effect = TRUE,
                              balanced_pleio = TRUE,
                              InSIDE_satisfied = TRUE,
                              rand_error = TRUE,     # remove random errors, for testing
                              two_sample = TRUE,     # 1- or 2-sample MR toggle, for testing
                              beta_val = 0.1,        # size of causal effect
                              allele_freq_min = 0.01,# frequency of effect allele
                              allele_freq_max = 0.99,
                              gamma_min = 0.03,      # size of pleiotropic effects on exposure
                              gamma_max = 0.1,
                              alpha_min = -0.2,      # size of pleiotropic effects on outcome
                              alpha_max = 0.2,
                              phi_min = -0.2,        # size of additional pleiotropic effects
                              phi_max = 0.2){        # when InSIDE not satisfied



  # Initialise blank lists to receive datasets for
  # each of:
  #     U (vector: unmeasured confounding exposures per participant),
  #     X (vector: exposure:outcome associations estimated per participant)
  #     Y (vector: gene:outcome association estimated per participant),
  #     G (Matrices: Genotype data)
  #
  #     gamma (vector: pleiotropic effects of each instrument on exposure)
  #     alpha (vector: pleiotropic effects of each instrument on outcome)
  #     phi (vector: additional pleiotropic effects of each instrument when InSIDE
  #     assumption not satisfied)
   U_list <- list()
   X_list <- list()
   Y_list <- list()
   G_X_list <- list()
   G_Y_list <- list()

   gamma_list <- list()
   alpha_list <- list()
   phi_list <- list()
   beta_list <- list()
   prop_invalid_list <- list()
```

```r
# --- Assign features common to all datasets ---#

 beta <- if_else(causal_effect == TRUE,# size of causal effect
                 beta_val,
                 0)

# create vector of participant indices for 1st n participants
# i.e. participants used for estimating gene:exposure coefficient
 sample_1_ref <- 1:n_participants


# Default is to estimate gene:outcome coefficient from different sample
# to gene:exposure coefficient (i.e. simulating 2-sample MR)
# two_sample == FALSE toggles to single sample for testing simulation

 ifelse(two_sample == FALSE,
        sample_2_ref <- sample_1_ref,# 1 sample MR
        sample_2_ref <- (n_participants+1):(2*n_participants))# 2 sample MR

# --- Create separate datasets ---#

# Create N datasets by simulating genotype matrices with
# 1 row per participant, 1 column per genetic instrument
# Use these to estimate U, X + Y

 for(n in 1:n_datasets){

  # Create error terms for U, X + Y per participant,
  # each drawn from standard normal distribution
  # unless random error turned off (for testing)

   ifelse(rand_error == TRUE,
          U_epsilon_vect <- rnorm(n = 2 * n_participants),
          U_epsilon_vect <- rep(0, 2 * n_participants))

   ifelse(rand_error == TRUE,
          X_epsilon_vect <- rnorm(n = n_participants),
          X_epsilon_vect <- rep(0, n_participants))

   ifelse(rand_error == TRUE,
          Y_epsilon_vect <- rnorm(n = n_participants),
          Y_epsilon_vect <- rep(0, n_participants))

  # --- Create matrix of genotypes ---#

  # 0 = reference, i.e. zero effect alleles,
  # 1 = 1 effect allele, 2 = 2 effect alleles

  # Probability of effect allele set per dataset
  # for each instrument, default value set at
  # random between 0.01-0.99 (i.e. both effect +
  # reference are common alleles)
```

```
 allele_freq_vect <- runif(n = n_instruments,
                           min = allele_freq_min,
                           max = allele_freq_max)

# Assign genotypes by sampling from binomial distribution
# twice (as two alleles) per participant with probability
# equal to frequency of effect allele
# Create twice as many genotypes as participants in sample
# to simulate 2 sample MR, i.e. first half used to estimate
# Gene:Exposure, second half used to estimate Gene:Outcome

# Matrix where columns are instruments, rows are participants
# Values 0, 1 or 2
 G_mat <- matrix(rbinom(n = 2 * n_participants * n_instruments,
                        size = 2,
                        prob = rep(allele_freq_vect, 2 * n_participants)),
                 nrow = 2 * n_participants,
                 ncol = n_instruments,
                 byrow = TRUE)

# --- Set characteristics for each genetic instrument ---#

# Set which instruments invalid, 0 = valid, 1 = invalid
 invalid_instrument_vect <- rbinom(n = n_instruments,
                                   size = 1,
                                   prob = prop_invalid)

# Set genetic effects of each instrument on the exposure,
# drawn from uniform distribution, min/max as per Bowden
# et al
 gamma_vect <- runif(n = n_instruments,
                     min = gamma_min,
                     max = gamma_max)

# Set pleiotropic effects on outcome, Scenarios and
# min/max from Bowden et al
 alpha_vect <- double()# Pleiotropic effects of instruments on outcome
 phi_vect <- double()# Pleiotropic effects of confounders on outcome

 for(j in 1:n_instruments){
   ifelse(invalid_instrument_vect[j] == 0,# alpha = 0 if valid
          alpha_vect[j] <- 0,
          ifelse(balanced_pleio == TRUE,
                 alpha_vect[j] <- runif(n = n_instruments,# balanced
                                        min = alpha_min,
                                        max = alpha_max),
                 alpha_vect[j] <- runif(n = n_instruments,# directional
                                        min = 0,
                                        max = alpha_max)
          )
   )

  # Assign default phi = 0 unless directional pleiotropy &
```

```
    # InSIDE assumption not satisfied & genetic instrument invalid
    if(balanced_pleio == FALSE & InSIDE_satisfied == FALSE){
      ifelse(invalid_instrument_vect[j] == 0,
             phi_vect[j] <- 0,
             phi_vect[j] <- runif(n = 1,
                                  min = phi_min,
                                  max = phi_max)
      )

    }
    else{
      phi_vect[j] <- 0
    }
  }

# --- Combine Gene matrix/parameters to recreate model ---#

# Create vectors of estimates for U, X and Y per individual,
# i.e. Ui, Xi and Yi. Uses matrix inner product operator " %*%"
# https://stackoverflow.com/questions/22060515/the-r-operator
# http://matrixmultiplication.xyz/

 Ui_vect <-  G_mat %*% phi_vect + U_epsilon_vect

 Xi_vect <-  G_mat[sample_1_ref, ] %*% gamma_vect +
   Ui_vect[sample_1_ref, ] +
   X_epsilon_vect

 Yi_vect <-  G_mat[sample_2_ref, ] %*% alpha_vect +
   beta * Xi_vect +
   Ui_vect[sample_2_ref, ] +
   Y_epsilon_vect

# Add vectors of estimates from this dataset to lists of
# estimates from all datasets
 U_list[[n]] <- Ui_vect

 X_list[[n]] <- Xi_vect

 Y_list[[n]] <- Yi_vect

 G_X_list[[n]] <- G_mat[sample_1_ref, ]

 G_Y_list[[n]] <- G_mat[sample_2_ref, ]

# Include actual parameters used in simulation for testing
 alpha_list[[n]] <- alpha_vect

 gamma_list[[n]] <- gamma_vect

 phi_list[[n]] <- phi_vect

 beta_list[[n]] <- beta
```

```
    prop_invalid_list[[n]] <- prop_invalid


  }

  #     U (vector: unmeasured confounding exposures per participant),
  #     X (vector: exposure:outcome associations estimated per participant)
  #     Y (vector: gene:outcome association estimated per participant)


  # --- Combine all outputs to return ---#

   combined_list <- list(U = U_list,        # Estimates
                         X = X_list,
                         Y = Y_list,
                         G_X = G_X_list,    # Genotypes of 1st sample
                         G_Y = G_Y_list,    # Genotypes of 2nd sample
                         alpha = alpha_list,# Actual values for validating simulation
                         gamma = gamma_list,
                         phi = phi_list,
                         beta = beta_list,
                         prop_invalid = prop_invalid_list
   )

   return(combined_list)
 }
```

This initial simulation function generated data in the following format:

```
# Check data produced in expected format
 set.seed(1701)
 test_data_sim <- simulate_MR_data(n_participants = 1000,
                                   n_instruments = 25,
                                   n_datasets = 2,
                                   prop_invalid = 0.3,
                                   rand_error = FALSE,
                                   causal_effect = TRUE,
                                   balanced_pleio = TRUE,
                                   InSIDE_satisfied = TRUE)

 str(test_data_sim)
```

```
## List of 10
##  $ U           :List of 2
##   ..$ : num [1:2000, 1] 0 0 0 0 0 0 0 0 0 0 ...
##   ..$ : num [1:2000, 1] 0 0 0 0 0 0 0 0 0 0 ...
##  $ X           :List of 2
##   ..$ : num [1:1000, 1] 1.12 1.59 1.76 1.49 1.56 ...
##   ..$ : num [1:1000, 1] 1.84 1.7 1.6 1.66 1.5 ...
##  $ Y           :List of 2
##   ..$ : num [1:1000, 1] -0.24 -0.311 -0.393 -0.227 -0.1 ...
##   ..$ : num [1:1000, 1] -0.872 -0.901 -0.772 -0.999 -0.477 ...
##  $ G_X         :List of 2
```

```
##   ..$ : int [1:1000, 1:25] 0 1 1 1 1 0 0 0 0 0 ...
##   ..$ : int [1:1000, 1:25] 1 2 1 2 2 2 2 2 2 2 ...
##  $ G_Y         :List of 2
##   ..$ : int [1:1000, 1:25] 0 1 1 0 1 0 0 0 0 0 ...
##   ..$ : int [1:1000, 1:25] 2 2 2 2 1 2 1 1 2 1 ...
##  $ alpha       :List of 2
##   ..$ : num [1:25] -0.106 0 -0.121 0 0 ...
##   ..$ : num [1:25] 0 0 -0.0786 0 0 ...
##  $ gamma       :List of 2
##   ..$ : num [1:25] 0.0902 0.0878 0.08 0.0832 0.084 ...
##   ..$ : num [1:25] 0.0374 0.0721 0.0975 0.085 0.0322 ...
##  $ phi         :List of 2
##   ..$ : num [1:25] 0 0 0 0 0 0 0 0 0 0 ...
##   ..$ : num [1:25] 0 0 0 0 0 0 0 0 0 0 ...
##  $ beta        :List of 2
##   ..$ : num 0.1
##   ..$ : num 0.1
##  $ prop_invalid:List of 2
##   ..$ : num 0.3
##   ..$ : num 0.3
```

A function (`extract_models`) was then written to create linear models from each dataset generated as per Bowden et al:

```
# Create plotting tibble with Mean/SD X + Y grouped by
# Dataset + instrument
 extract_models <- function(sim){

   output_list <- list()

  # Create linear models per dataset to get coefficients
  # for gene:exposure association (coeff_G_X) and gene:outcome
  # association (coeff_G_Y)
   for(dataset in 1:length(sim$X)){

     X <- sim$X[[dataset]]
     Y <- sim$Y[[dataset]]
     Instruments_X <- sim$G_X[[dataset]]
     Instruments_Y <- sim$G_Y[[dataset]]

     alpha <- sim$alpha[[dataset]]
     gamma <- sim$gamma[[dataset]]
     phi <- sim$phi[[dataset]]
     beta <- sim$beta[[dataset]]
     prop_invalid <- sim$prop_invalid[[dataset]]

    # Model for gene:exposure
     X_lm <- lm(X ~ 0 + Instruments_X)
     coeff_G_X_vect <- coef(summary(X_lm))[1:(ncol(Instruments_X)), 1]
     SE_coeff_G_X_vect <- coef(summary(X_lm))[1:(ncol(Instruments_X)), 2]

    # Model for gene:outcome
     Y_lm <- lm(Y ~ 0 + Instruments_Y)
     coeff_G_Y_vect <- coef(summary(Y_lm))[1:(ncol(Instruments_Y)), 1]
```

```
    SE_coeff_G_Y_vect <- coef(summary(Y_lm))[1:(ncol(Instruments_Y)), 2]

    output_list[[dataset]] <- as_tibble(list(dataset = dataset,
                                             Instrument = c(1:ncol(Instruments_X)),
                                             coeff_G_X = coeff_G_X_vect,
                                             coeff_G_X_SE = SE_coeff_G_X_vect,
                                             gamma = gamma,
                                             coeff_G_Y = coeff_G_Y_vect,
                                             coeff_G_Y_SE = SE_coeff_G_Y_vect,
                                             alpha = alpha,
                                             phi = phi,
                                             beta = beta,
                                             prop_invalid = prop_invalid),
                                        .name_repair = "unique")
  }

  return(output_list)

}
```

These models generated estimates of the coefficient of gene:exposure association (`coeff_G_X`), coefficient of gene:outcome association (`coeff_G_Y`), and the elevant standard errors of these estimates. The values of parameters inputted were also returned to aid in further testing of data/model generation, i.e. actual ene:exposure associations (`gamma`), pleiotropic effects of invalid instruments (`alpha`), additional pleiotropic effects when InSIDE assumption not satified `phi`), causal effect of exposure on outcome (`beta`) and the proportion of invalid genetic instruments with pleiotropic effects on the outcome (`prop_invalid`).

```
test_extract_model <- extract_models(test_data_sim)

summary(test_extract_model[[1]])
```

```
##     dataset    Instrument   coeff_G_X        coeff_G_X_SE
##  Min.   :1   Min.   : 1   Min.   :0.03006   Min.   :1.591e-16
##  1st Qu.:1   1st Qu.: 7   1st Qu.:0.03791   1st Qu.:1.702e-16
##  Median :1   Median :13   Median :0.05578   Median :1.847e-16
##  Mean   :1   Mean   :13   Mean   :0.06018   Mean   :2.346e-16
##  3rd Qu.:1   3rd Qu.:19   3rd Qu.:0.07998   3rd Qu.:2.441e-16
##  Max.   :1   Max.   :25   Max.   :0.09140   Max.   :7.259e-16
##      gamma           coeff_G_Y           coeff_G_Y_SE          alpha
##  Min.   :0.03006   Min.   :-0.1188256   Min.   :0.0009824   Min.   :-0.120669
##  1st Qu.:0.03791   1st Qu.: 0.0006676   1st Qu.:0.0010520   1st Qu.: 0.000000
##  Median :0.05578   Median : 0.0031161   Median :0.0011837   Median : 0.000000
##  Mean   :0.06018   Mean   :-0.0047291   Mean   :0.0014576   Mean   :-0.008692
##  3rd Qu.:0.07998   3rd Qu.: 0.0068099   3rd Qu.:0.0015114   3rd Qu.: 0.000000
##  Max.   :0.09140   Max.   : 0.1356693   Max.   :0.0040567   Max.   : 0.133513
##       phi         beta      prop_invalid
##  Min.   :0   Min.   :0.1   Min.   :0.3
##  1st Qu.:0   1st Qu.:0.1   1st Qu.:0.3
##  Median :0   Median :0.1   Median :0.3
##  Mean   :0   Mean   :0.1   Mean   :0.3
##  3rd Qu.:0   3rd Qu.:0.1   3rd Qu.:0.3
##  Max.   :0   Max.   :0.1   Max.   :0.3
```

B.0.2 Testing Generation of Data and Models

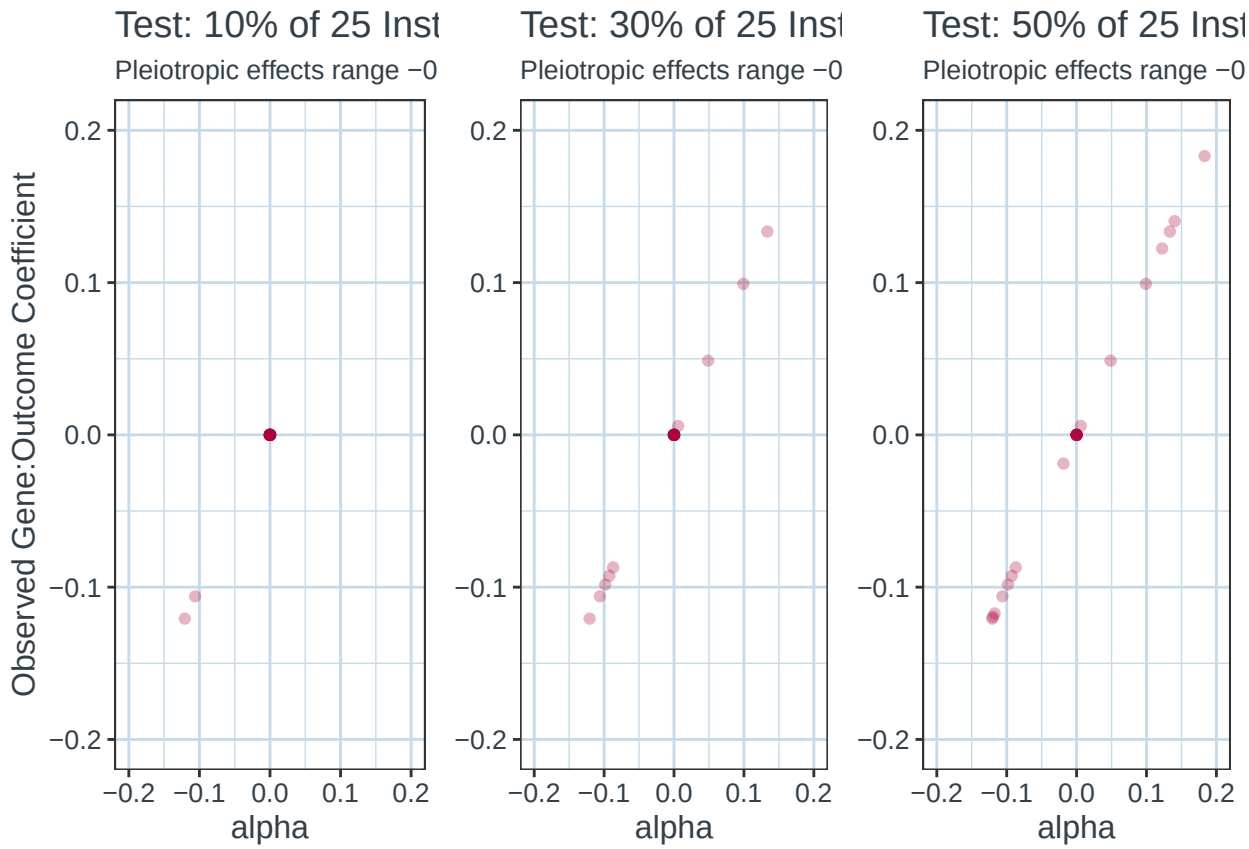
A series of test plots were used to verify that data were simulated as intended under the various conditions specified by input parameters. Test plots were not reated for the parameters `n_participants`, `n_instruments` or `n_datasets`, as the functioning of these parameters could be readily inferred from the structure of he datasets outputted, as above.

The `prop_invalid` parameter specifies the proportion of invalid genetic instruments simulated, i.e. the proportion of genetic instruments affecting the outcome via irect/pleiotropic effects, and thus not solely via the exposure of interest. If simulated correctly, increasing the value of `prop_invalid` should increase the umber of instruments with pleiotropic effects, i.e. instruments with `alpha` $=/= 0$. With random error terms set to 0 and no causal effect present (i.e. `rand_error = ALSE` and `causal_effect = FALSE`), the estimated gene:outcome coefficient estimated using any given instrument will equal the pleiotropic effects of that instrument i.e. `coeff_G_Y = alpha`), and therefore will only be non-zero for invalid instruments with non-zero pleiotropic effects on the outcome . Plotting `coeff_G_Y` gainst `alpha` for simulated data with no causal effect or random error should therefore yield a graph where

- For valid instruments: gene:outcome coefficient = alpha = 0
- For invalid instruments: gene:outcome coefficient = alpha $=/= 0$, with values spread uniformly between `alpha_min` and `alpha_max`

```
# Check altering proportion of invalid instruments alters
# proportion of instruments displaying pleiotropic effects
# N.B. cluster around alpha = 0 represents valid instruments with
# no pleiotropic effects

# 10% of instruments invalid
 set.seed(1701)
 sim_test_data_inval_0.1 <- simulate_MR_data(n_participants = 1000,
                                             n_instruments = 25,
                                             n_datasets = 1,
                                             prop_invalid = 0.1,
                                             rand_error = FALSE,
                                             causal_effect = FALSE,
                                             alpha_min = -0.2,
                                             alpha_max = 0.2)

# 30% of instruments invalid
 set.seed(1701)
 sim_test_data_inval_0.3 <- simulate_MR_data(n_participants = 1000,
                                             n_instruments = 25,
                                             n_datasets = 1,
                                             prop_invalid = 0.3,
                                             rand_error = FALSE,
                                             causal_effect = FALSE,
                                             alpha_max = 0.2)

# 50% of instruments invalid
 set.seed(1701)
 sim_test_data_inval_0.5 <- simulate_MR_data(n_participants = 1000,
                                             n_instruments = 25,
                                             n_datasets = 1,
                                             prop_invalid = 0.5,
                                             rand_error = FALSE,
```

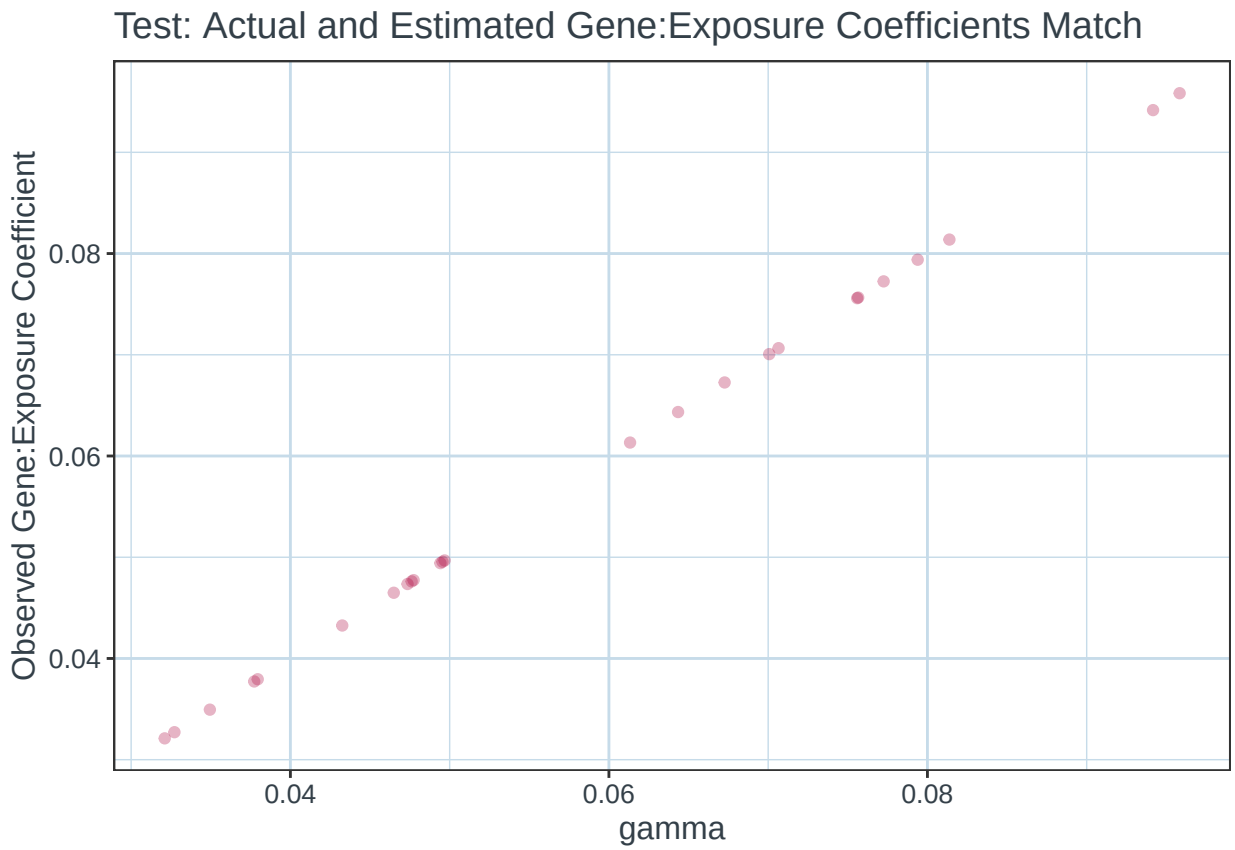
```
                                          causal_effect = FALSE,
                                          alpha_min = -0.2,
                                          alpha_max = 0.2)


test_plot_tib_inval_0.1 <- extract_models(sim_test_data_inval_0.1)[[1]]
test_plot_tib_inval_0.3 <- extract_models(sim_test_data_inval_0.3)[[1]]
test_plot_tib_inval_0.5 <- extract_models(sim_test_data_inval_0.5)[[1]]

test_plot_inval_0.1 <- test_plot_tib_inval_0.1 %>%
  select(alpha, coeff_G_Y) %>%
  plot_template() +
  geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
  aes(x = alpha, y = coeff_G_Y ) +
  scale_y_continuous(limits = c(-0.2, 0.2)) +
  scale_x_continuous(limits = c(-0.2, 0.2)) +
  labs(y = "Observed Gene:Outcome Coefficient",
       title = "Test: 10% of 25 Instruments Invalid",
       subtitle = "Pleiotropic effects range -0.2 to 0.2")

test_plot_inval_0.3 <- test_plot_tib_inval_0.3 %>%
  select(alpha, coeff_G_Y) %>%
  plot_template() +
  geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
  aes(x = alpha, y = coeff_G_Y ) +
  scale_y_continuous(limits = c(-0.2, 0.2)) +
  scale_x_continuous(limits = c(-0.2, 0.2)) +
  labs(y = "Observed Gene:Outcome Coefficient",
       title = "Test: 30% of 25 Instruments Invalid",
       subtitle = "Pleiotropic effects range -0.2 to 0.2") +
  theme(axis.title.y = element_blank())

test_plot_inval_0.5 <- test_plot_tib_inval_0.5 %>%
  select(alpha, coeff_G_Y) %>%
  plot_template() +
  geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
  aes(x = alpha, y = coeff_G_Y ) +
  scale_y_continuous(limits = c(-0.2, 0.2)) +
  scale_x_continuous(limits = c(-0.2, 0.2)) +
  labs(y = "Observed Gene:Outcome Coefficient",
       title = "Test: 50% of 25 Instruments Invalid",
       subtitle = "Pleiotropic effects range -0.2 to 0.2") +
  theme(axis.title.y = element_blank())

plot_grid(test_plot_inval_0.1,
          test_plot_inval_0.3,
          test_plot_inval_0.5,
          ncol = 3,
          rel_widths = c(1.1, 1, 1))
```



Similarly, with random error terms set to 0 and no causal effect present, gene:exposure coefficients estimated for each instrument should exactly match the actual alues simulated, i.e. `coeff_G_X = gamma` for all instruments:

```
# Check observed gene:exposure coefficients for each instrument
# (coeff_G_X) approximate true values (gamma) when a causal effect
# is present & a large number of participants are included
 set.seed(1701)
 sim_test_data_gamma_1 <- simulate_MR_data(n_participants = 100,
                                           n_instruments = 25,
                                           n_datasets = 1,
                                           prop_invalid = 0.1,
                                           causal_effect = FALSE,
                                           rand_error = FALSE,
                                           balanced_pleio = TRUE,
                                           InSIDE_satisfied = TRUE)


 test_plot_tib_gamma_1 <- extract_models(sim_test_data_gamma_1)[[1]]

 test_plot_tib_gamma_1 %>%
   select(gamma, coeff_G_X) %>%
   plot_template() +
   geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
   aes(x = gamma, y = coeff_G_X ) +
   labs(y = "Observed Gene:Exposure Coefficient",
```

```
        title = "Test: Actual and Estimated Gene:Exposure Coefficients Match")
```

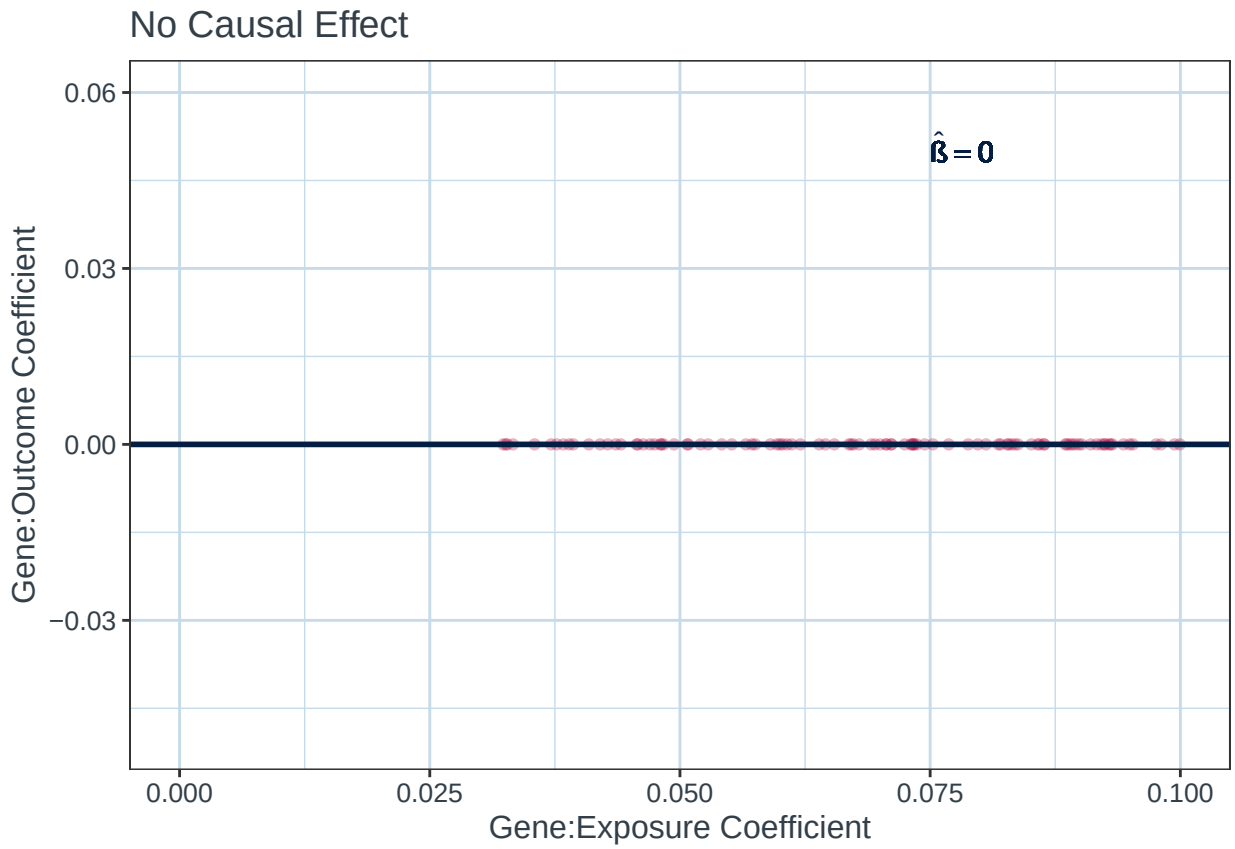


For the next phase of testing, a function (`plot_GY_GX`) was written to plot the coefficients for gene:exposure versus gene:outcome as estimated using the reviously created linear models:

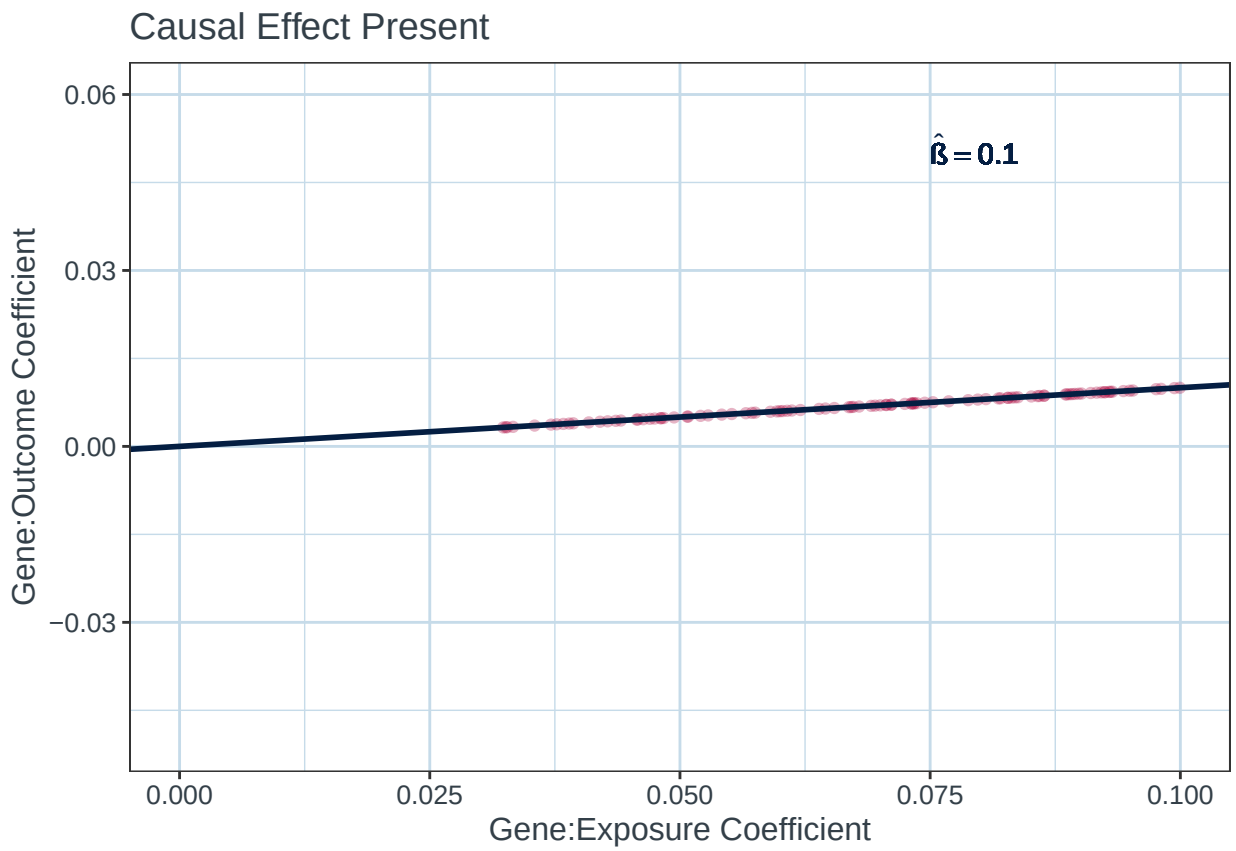
```
plot_GY_GX <- function(model_tib,
                       plot_title = as.character(NA),
                       x_min = 0,                    # set x-axis limits
                       x_max = 0.1,
                       y_min = -0.05,                # set x-axis limits
                       y_max = 0.06,
                       beta_x = 0.075,               # set beta-hat position
                       beta_y = 0.05,
                       hat_offset = 0.003
                       )
  {

  model_tib %>%
    mutate(Gradient = round(coefficients(lm(coeff_G_Y ~ 0 + coeff_G_X, digits = 2))[1], 5)) %>%
    plot_template() +
    aes(x = coeff_G_X, y = coeff_G_Y) +
    geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
    geom_abline(aes(intercept = 0,
                    slope = Gradient),
                size = 1,
                colour = edin_uni_blue_hex) +
```

```
    geom_text(aes(x = beta_x,# labels with gradient (causal effect estimate)
                  y = beta_y,
                  label = paste0("\U03B2 == ", as.character(Gradient))),#beta
              colour = edin_uni_blue_hex,
              hjust = 0,
              parse = TRUE) +
    annotate("text",
             x = beta_x,     # add hat to beta
             y = beta_y + hat_offset,
             label = paste("\U02C6"),
             colour = edin_uni_blue_hex,
             hjust = -0.4,
             vjust = 0.9,
             parse = TRUE) +
    labs(title = plot_title,
         x = "Gene:Exposure Coefficient",
         y = "Gene:Outcome Coefficient") +
    xlim(x_min, x_max) +
    ylim(y_min, y_max)

}
```

```
# No causal effect present
 set.seed(1701)
 sim_test_data_causal_0 <- simulate_MR_data(n_participants = 1000,
                                            n_instruments = 100,
                                            n_datasets = 1,
                                            prop_invalid = 0,
                                            causal_effect = FALSE,
                                            rand_error = FALSE)

 test_plot_tib_causal_0 <- extract_models(sim_test_data_causal_0)[[1]]

 plot_GY_GX(test_plot_tib_causal_0, plot_title = "No Causal Effect")
```

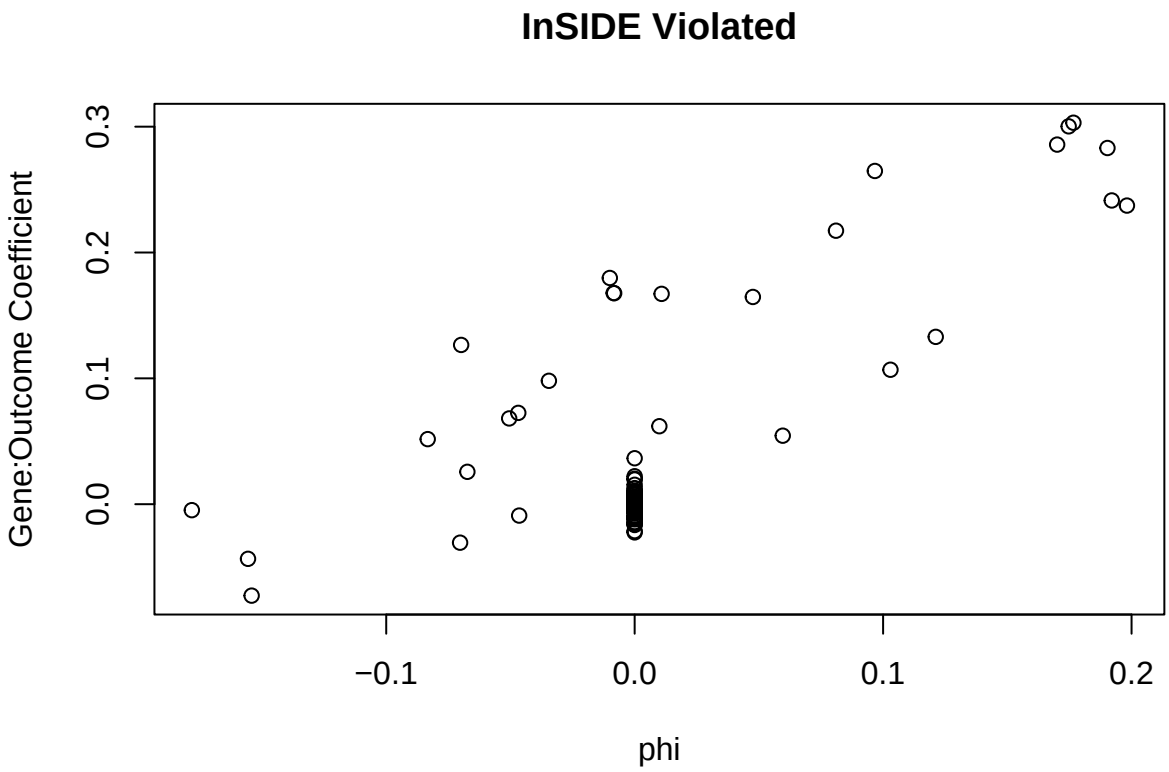



```r
# Check altering proportion of invalid instruments alters
# proportion of instruments displaying pleiotropic effects
# N.B. cluster around alpha = 0 represents valid instruments with
# no pleiotropic effects


# Causal effect present
 set.seed(1701)
 sim_test_data_causal_1 <- simulate_MR_data(n_participants = 1000,
                                            n_instruments = 100,
                                            n_datasets = 1,
                                            prop_invalid = 0,
                                            causal_effect = TRUE,
                                            rand_error = FALSE,
                                            two_sample = FALSE)

 test_plot_tib_causal_1 <- extract_models(sim_test_data_causal_1)[[1]]

 plot_GY_GX(test_plot_tib_causal_1, plot_title = "Causal Effect Present")
```

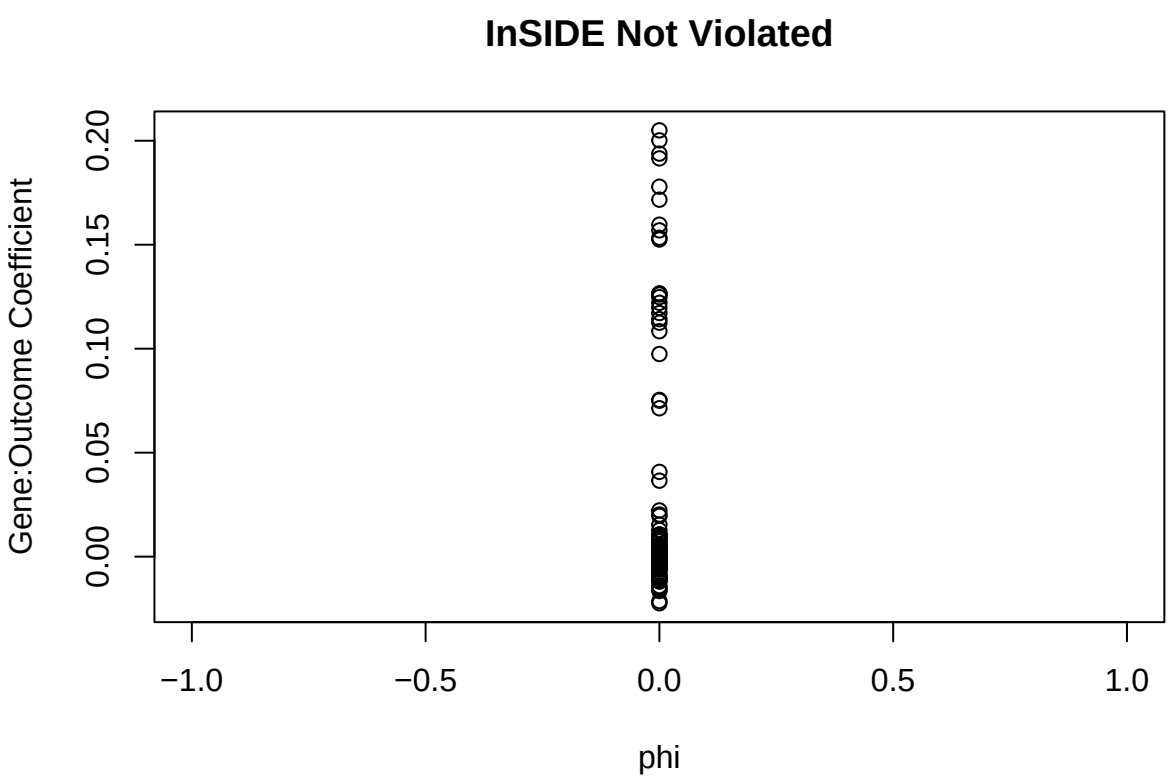



```
# Check violating InSIDE assumption results in distorted
# estimation of pleiotropic effects
# N.B. cluster around alpha = 0 represents valid instruments with
# no pleiotropic effects
 set.seed(1701)
 sim_test_data_phi_T <- simulate_MR_data(n_participants = 100000,
                                         n_instruments = 100,
                                         n_datasets = 1,
                                         prop_invalid = 0.3,
                                         causal_effect = FALSE,
                                         balanced_pleio = FALSE,
                                         InSIDE_satisfied = FALSE)

 set.seed(1701)
 sim_test_data_phi_F <- simulate_MR_data(n_participants = 100000,
                                         n_instruments = 100,
                                         n_datasets = 1,
                                         prop_invalid = 0.3,
                                         causal_effect = FALSE,
                                         balanced_pleio = FALSE,
                                         InSIDE_satisfied = TRUE)


 test_plot_tib_phi_T <- extract_models(sim_test_data_phi_T)[[1]]
 test_plot_tib_phi_F <- extract_models(sim_test_data_phi_F)[[1]]
```

```
test_plot_tib_phi_T %>%
  select(phi, coeff_G_Y) %>%
  plot(.,
       main = "InSIDE Violated",
       ylab = "Gene:Outcome Coefficient")
```




```
test_plot_tib_phi_F %>%
  select(phi, coeff_G_Y) %>%
  plot(.,
       main = "InSIDE Not Violated",
       ylab = "Gene:Outcome Coefficient")
```




```
#phi on y, not alpha
```

C Appendix C: Citation Search Strategy