

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

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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: 918

1 Appendices

1.1 Appendix A: List of Abbreviations

1.2 Appendix B: Simulation Code

```
knitr::opts_chunk$set(echo = TRUE,
                      warning=FALSE,
                      message=FALSE,
                      error=FALSE,
                      cache = TRUE)

# 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)
```

1.2.1 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:

“... ”

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

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

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

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

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 exposure 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 valid 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.” [?]

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 parameters 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()

# --- 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.  $U_i$ ,  $X_i$  and  $Y_i$ . 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 relevant 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 gene:exposure associations (`gamma`), pleiotropic effects of invalid instruments (`alpha`), additional pleiotropic effects when InSIDE assumption not satisfied (`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
```

1.2.2 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 created for the parameters `n_participants`, `n_instruments` or `n_datasets`, as the functioning of these parameters could be readily inferred from the structure of the 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 direct/pleiotropic effects, and thus not solely via the exposure of interest. If simulated correctly, increasing the value of `prop_invalid` should increase the number of instruments with pleiotropic effects, i.e. instruments with $\alpha \neq 0$. With random error terms set to 0 and no causal effect present (i.e. `rand_error` = `FALSE` 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` = α , and therefore will only be non-zero for invalid instruments with non-zero pleiotropic effects on the outcome. Plotting `coeff_G_Y` against α for simulated data with no causal effect or random error should therefore yield a graph where

- For valid instruments: gene:outcome coefficient = α = 0
- For invalid instruments: gene:outcome coefficient = $\alpha \neq 0$, 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]]

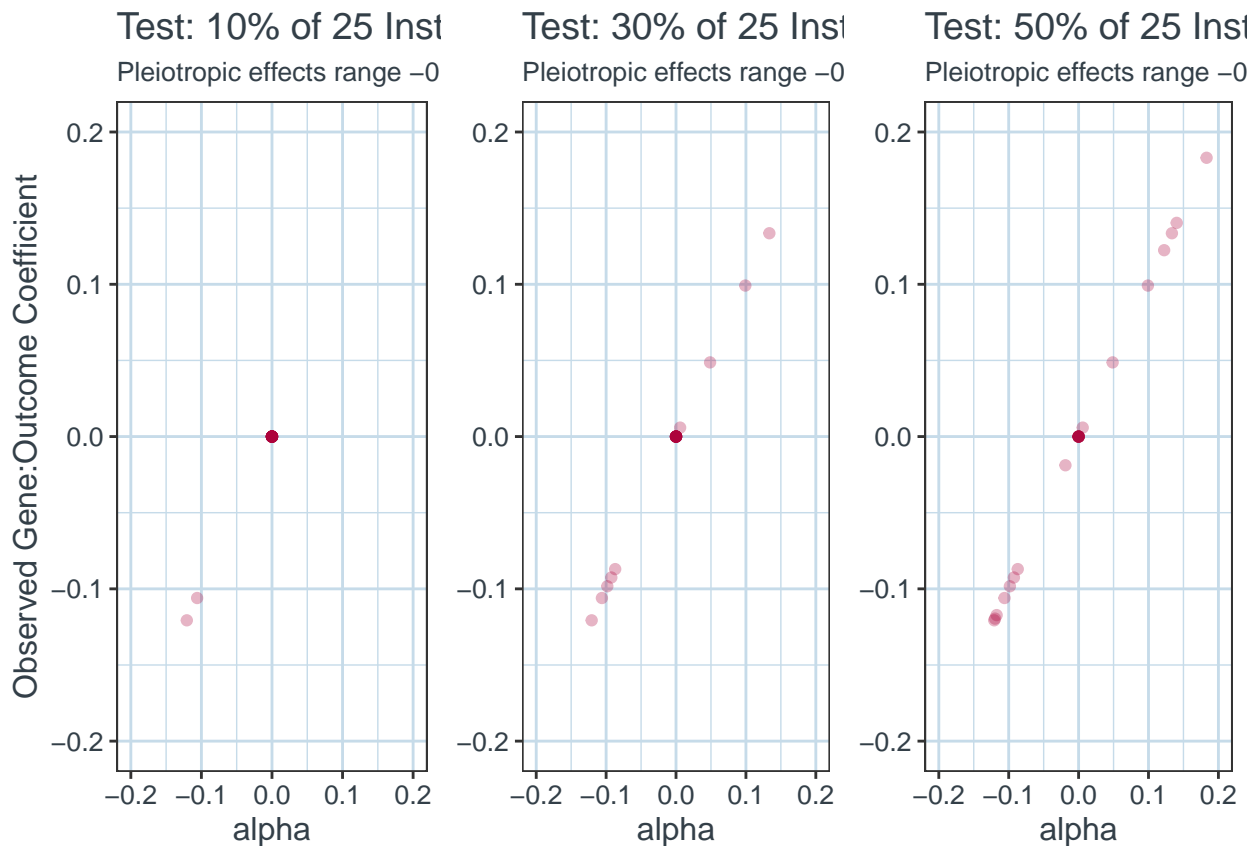
test_plot_inval_0.1 <- test_plot_tib_inval_0.1 %>%
  select(alpha, coeff_G_Y) %>%
  plot_template() +
  geom_point(colour = edin_bright_red_hex, alpha = 0.3) +
  aes(x = alpha, y = coeff_G_Y) +
  scale_y_continuous(limits = c(-0.2, 0.2)) +
  scale_x_continuous(limits = c(-0.2, 0.2)) +
  labs(y = "Observed 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))
```

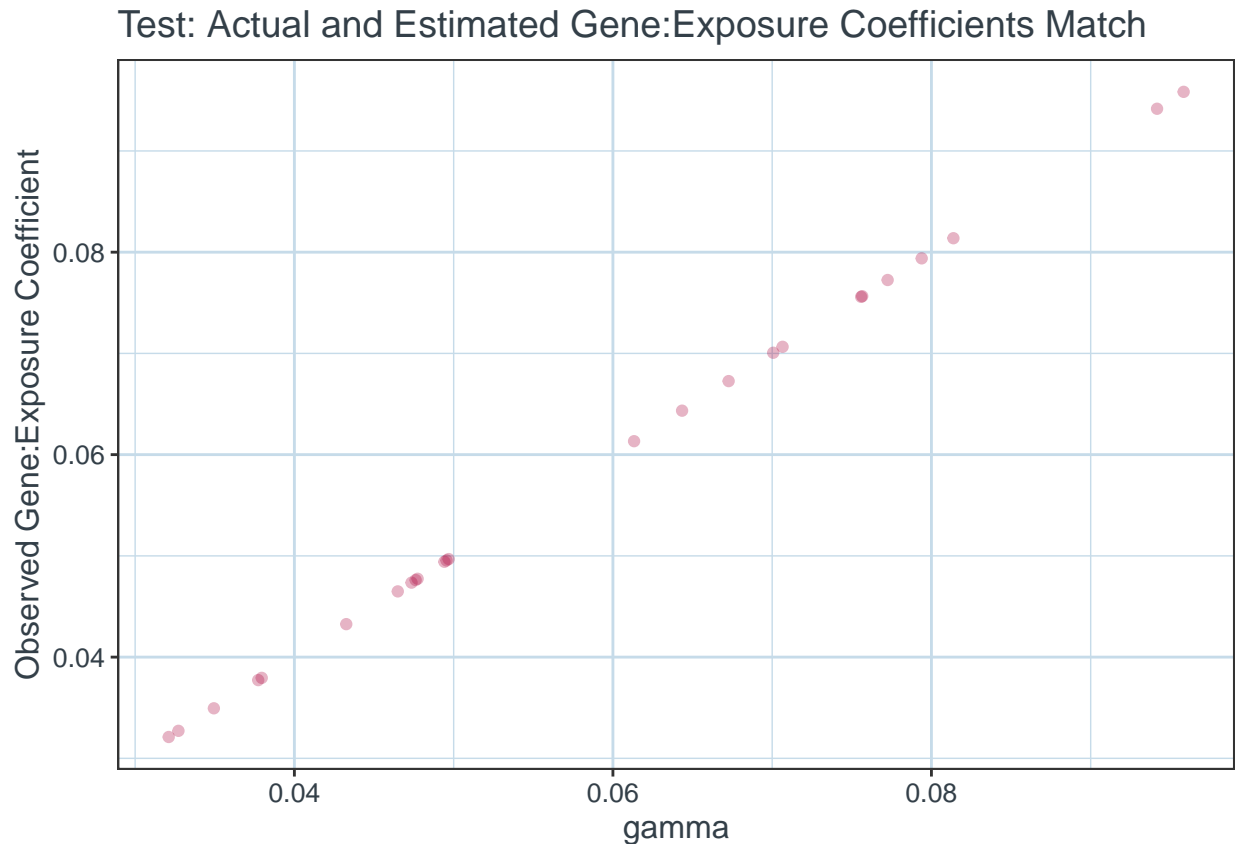


Similarly, with random error terms set to 0 and no causal effect present, gene:exposure coefficients estimated for each instrument should exactly match the actual values simulated, i.e. `coeff_G_X = gamma` for all instruments:

```
# 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 previously 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 y-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)
}

```

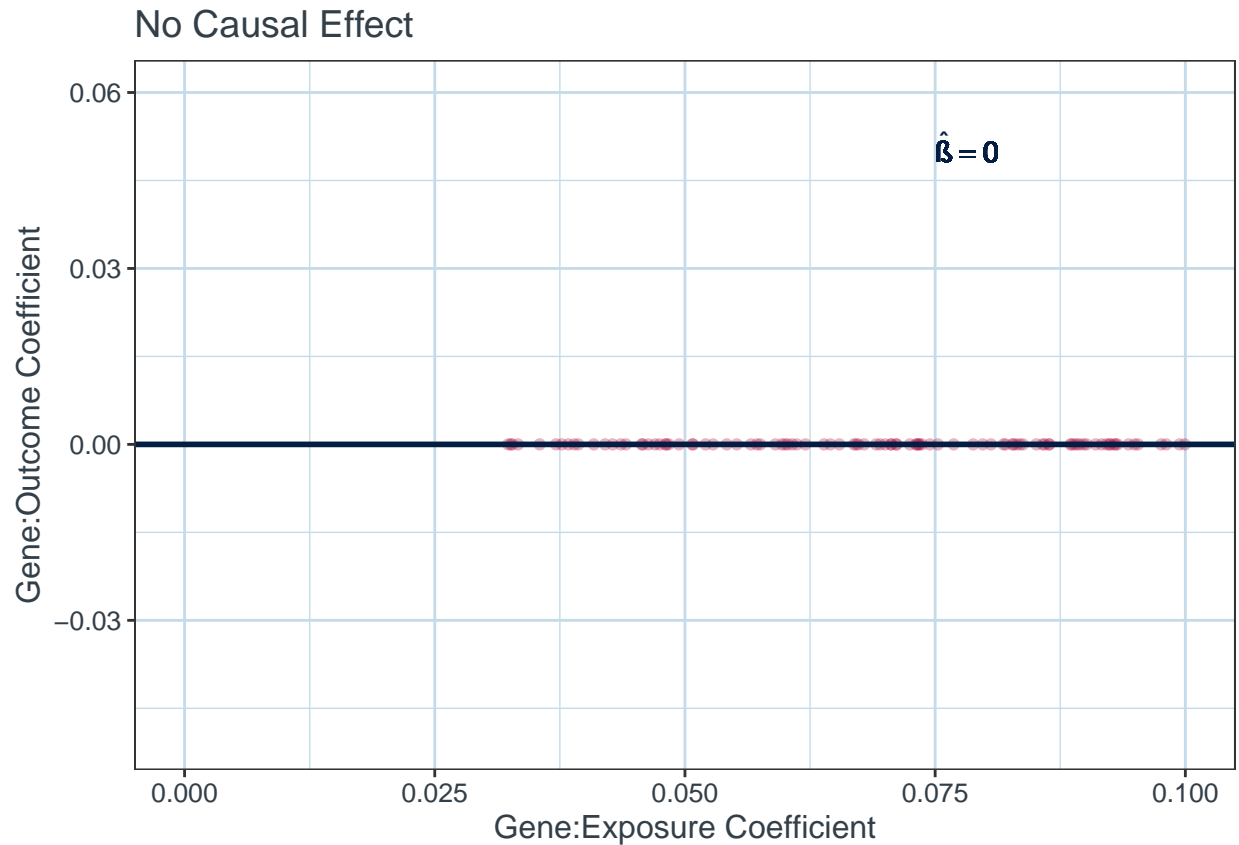
```

# 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")

```

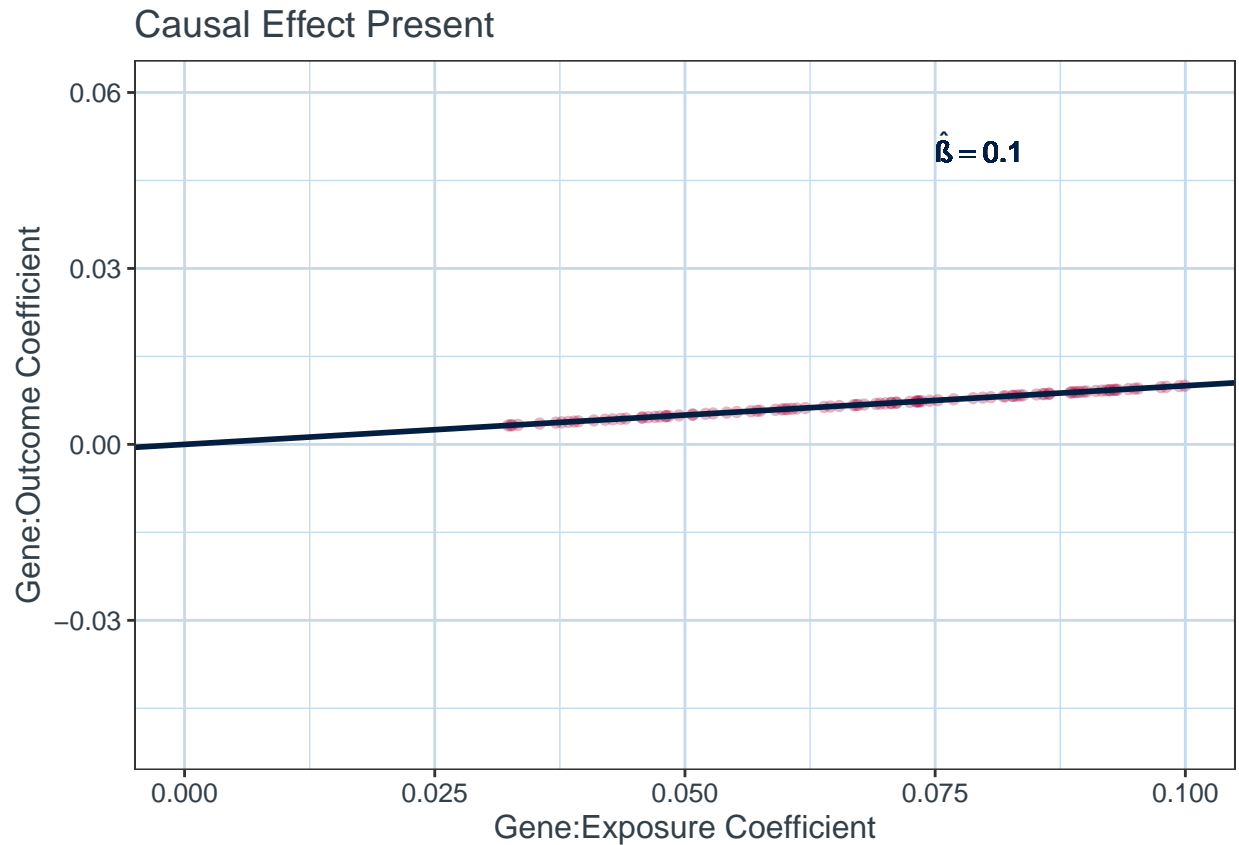


```
# Check altering proportion of invalid instruments alters
# proportion of instruments displaying pleiotropic effects
# N.B. cluster around alpha = 0 represents valid instruments with
# no pleiotropic effects

# 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")
```

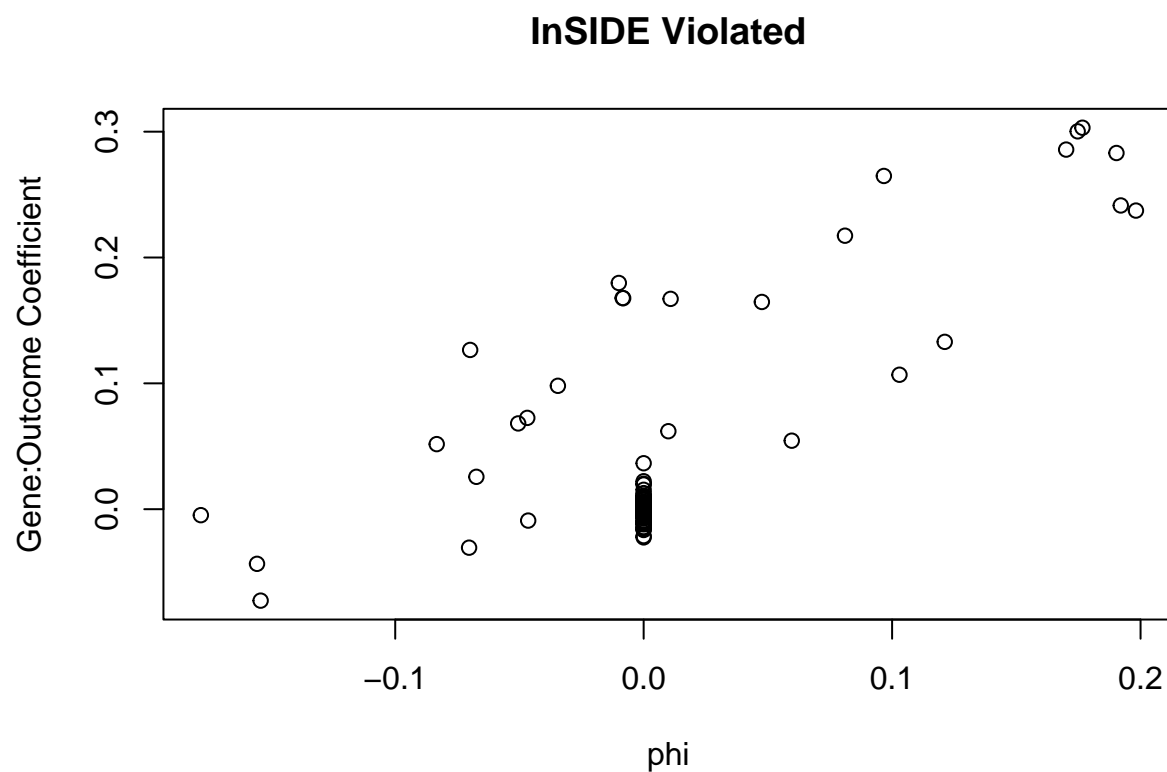



```
# Check violating InSIDE assumption results in distorted
# estimation of pleiotropic effects
# N.B. cluster around alpha = 0 represents valid instruments with
# no pleiotropic effects
set.seed(1701)
sim_test_data_phi_T <- simulate_MR_data(n_participants = 100000,
                                       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)

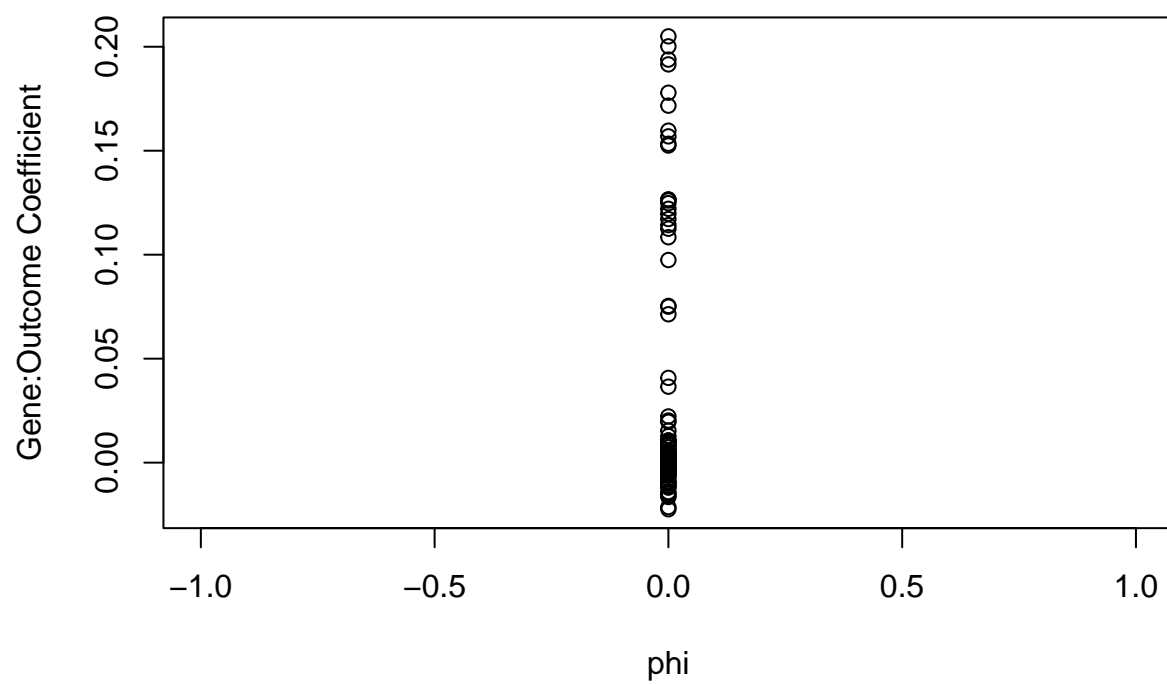
test_plot_tib_phi_T <- extract_models(sim_test_data_phi_T)[[1]]
test_plot_tib_phi_F <- extract_models(sim_test_data_phi_F)[[1]]
```

```
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")
```

InSIDE Not Violated



#phi on y, not alpha

1.3 Appendix C: Citation Search Strategy