

Towards the construction of a causal map of the human phenome

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Introduction

Mendelian randomisation (MR) (1,2) exploits genetic pleiotropy to infer the causal relationships between phenotypes. Suppose that one trait (the exposure) causally influences another (the outcome). If a SNP influences the outcome through the exposure then the SNP is exhibiting vertical pleiotropy. Such a genetic variant is considered to be an instrumental variable for the exposure, and can be exploited to mimic a randomised controlled trial, enabling a causal estimate to be made by comparing the outcome phenotypes between those individuals that have the exposure-increasing allele against those who do not. Multiple independent genetic variants for a particular exposure can be used jointly to improve causal inference, because a) each variant is an independent natural experiment, and an overall causal estimate can be obtained by meta-analysing the single estimates from each instrument; and b) some violations of the assumptions of MR (most notably that the SNP influences the outcome through no pathways other than the exposure) can be evaluated through sensitivity analyses.

Genome-wide association studies (GWAS) have identified genetic instrumental variables for thousands of phenotypes. Recent developments in Mendelian randomisation have enabled knowledge of instrumental variables to be applied using only summary level data (known as two-sample MR, 2SMR). Here, in order to infer the causal effect of an exposure on an outcome all that is required is an estimate of the genetic effects of the instrumenting SNP on the exposure, and the corresponding estimate of the effect on the outcome. This has two major advantages. First, GWAS summary data is non-disclosive and often publicly available. Second, causal inference can be made between phenotypes even if they have not been measured in the same samples, limiting the breadth of possible causal estimates only to the availability of GWAS summary data for the traits in question.

Problems with obtaining unbiased causal effects can arise, however, if the genetic instruments exhibit horizontal pleiotropy (HP), where they influence the outcome through a pathway other than the exposure. The extent of this problem is not to be understated, and many methods have been developed that attempt to reliably obtain unbiased causal estimates under specific models of HP. It is considered best practice to report estimates from all available methods as sensitivity analyses when presenting causal estimates, however this strategy is not necessarily optimal for several reasons. First, if different methods disagree it is not possible to know which is correct because the appropriate model of HP is not known. Second, though the IVW approach is most statistically powerful under no HP, it can have high false negative or low true positive rates in the presence of HP compared to other methods. Given that pleiotropy has been hypothesised to be universal, defaulting to the IVW method in the first instance and using other methods as sensitivity analyses may not be appropriate. Third, the available methods do not cover all possible models of HP, and therefore an automated method for instrument selection may be necessary. Fourth, it could be of interest to make causal effect estimates for thousands of traits, in which case a discerning evaluation of each causal effect of interest may not be possible or convenient.

In this paper we introduce two innovations towards improving the reliability of MR estimates. First, we introduce an approach to automatically discard genetic variants that are likely to be invalid. Second, we hypothesised that characteristics of the summary data could indicate which method would be most reliable, and we introduce new machine learning approaches that attempt to automate both instrument and method selection. Using curated GWAS summary data for thousands of phenotypes, we use these new methods to construct a graph of millions of causal estimates. We consider this to be only a ‘first draft’ of the causal map of the human phenome.

Methods

GWAS summary data and their use in 2SMR

The use of summary data in two-sample MR is described in detail elsewhere. A brief outline of the procedure is as follows. First, genetic instruments for the exposure trait need to be identified - those SNPs with $p < 10^{-8}$ are retained, collecting their effect sizes and standard errors and effect alleles for the association with the exposure trait. These can be obtained manually from complete summary data or from curated lists of significant GWAS associations. Next, the effects of those SNPs on the outcome need to be obtained, typically necessitating complete summary data because it is unlikely that these SNPs will have reached genome-wide significance (and therefore be present in curated catalogues). At its most simple implementation, the regression of the SNP-exposure effect sizes against the SNP-outcome effect sizes, with greater weight afforded to those SNPs with smaller SNP-outcome standard errors, provides the estimate of the causal effect of the exposure on the outcome.

Given summary data for a large number of traits, it is straightforward to exhaustively analyse the causal relationships of every trait against every other trait for which there is sufficient summary data available. Supplementary table X provides a list of all traits that have available GWAS summary data, indicating if they have complete summary data (in which case they can be used as both exposure traits and outcome traits), or if they only have significant associations (in which they can only be used as exposure traits). Supplementary table X provides a list of the genetic instruments used for each trait.

Mendelian randomisation methods and their assumptions

In this paper we consider three main classes of MR estimation. Full details for each approach have been described extensively elsewhere.

Mean-based methods: Here we consider four nested models. The inverse variance weighted (IVW) fixed effects meta-analysis approach assumes that variants exhibit no HP. IVW random effects meta-analysis relaxes the HP assumption, allowing it to be present but balanced - such that it only leads to increased heterogeneity around the regression and not introducing bias. Fixed effects Egger regression relaxes the HP assumption further by allowing a non-zero intercept which essentially allows horizontal pleiotropy be directional, where it systematically occurs in a specific direction. Random effects Egger regression allows heterogeneity around the directional HP, as long as the HP effects are not correlated with the SNP-exposure effects (this is the INSIDE assumption).

The Rucker framework uses estimates of heterogeneity in the IVW and Egger frameworks to navigate between these nested models. A jackknife approach (random selection with replacement of instruments) can be used to obtain a sampling distribution for the model estimate amongst these four variations. Using 1000 rounds of jackknife estimates, we can obtain a final estimate using the mean or the median of the distribution. We only use the jackknife approach for associations where there are 15 or more variants to avoid combinatorial saturation.

Median-based methods: An alternative approach is to take the median effect of all available instruments. This has the advantage that up to half the instruments can be invalid, and the estimate will remain unbiased. Developing the approach further to allow stronger instruments to contribute more towards the estimate can be obtained by obtaining the median of the weights of each instrument. The penalised weighted median estimator ...

Mode-based methods: Similar to the median, the mode based estimator clusters the instruments into groups based on similarity of causal effects, and returns the final causal effect estimate based on the cluster that has the largest number of instruments. This can be extended in two ways, first by weighting the strength of each cluster by the inverse of the variance of the SNP-outcome associations in the cluster; second by weighting the strength of each cluster by the inverse of the variance of the SNP-exposure associations.

Instrument selection

Top hits

The simplest approach to selecting instruments for performing MR is to take SNPs that have been declared significant in the published GWAS for the exposure. This typically involves obtaining SNPs that surpass $p < 5 \times 10^{-8}$, using clumping to obtain independent SNPs, and then replicating in an independent sample. These results are often recorded in public GWAS catalogs. Alternatively the clumping procedure can be performed using complete summary data in MR-Base. We call this the “top hits” strategy.

Steiger filtering

With genome-wide association studies growing ever larger, the statistical power to detect significant associations that may be influencing the trait downstream of many other pathways increases. For example, if a SNP g_A influences trait A , and trait A influences trait B , then a sufficiently powered GWAS will identify the g_A as being significant for trait B (Figure 1a). Using g_A as an instrument to test the causal effect of A on B is perfectly valid. But in the (incorrectly hypothesised) MR analysis of trait B on trait A could erroneously result in the apparent causal association of B on A . If g_A is only one of many known instruments for B , amongst which some are valid, it is to the advantage of the researcher to exclude g_A from the analysis.

An approach to inferring the causal direction between phenotypes was developed recently, using the following basic premise. If trait A causes trait B then

$$\sum_{i=1}^M \text{cor}(g_i, A)^2 > \sum_{i=1}^M \text{cor}(g_i, B)^2$$

because the $\text{cor}(g_i, B)^2 = \text{cor}(A, B)^2 \text{cor}(g_i, A)^2$. This simple inequality will not hold in some cases, for example $\rho_{x, x_o} < \rho_{x, y} \rho_{y, y_o}$ where ρ_{x, x_o} and ρ_{y, y_o} are the precision of the measurements of the x and y . Steiger’s Z-test of correlated correlations can be used to formally test the extent to which the two correlations are statistically different.

Here we adapt this approach to automatically filter SNPs that are liable to be invalid (Figure 1a). In this case the Steiger test applied to each variant in turn will identify g_A as being unlikely to primarily associate with B relative to A . Similarly, for SNPs that influence confounders of A and B or those variants that exhibit horizontal pleiotropy, the difference in $\text{cor}(g_i, A)^2$ and $\text{cor}(g_i, B)^2$ will be reduced, increasing the likelihood of the SNP being excluded because the Steiger Z-test is less likely to be significant.

To estimate $\text{cor}(g, x)^2$, if x is continuous we obtain the F-statistic from the reported p-value and sample size and then $\text{cor}(g, x)^2 = \frac{F}{N-2-F}$. If x is binary then our objective is to estimate the variance of the risk liability explained by the SNP, $\text{cor}(g, x)^2 = \frac{V_a}{V_a + V_e}$. Here, $V_e = \pi^2/3$, and $V_a = 2\beta^2 p(1-p)$, where β is the log odds ratio and p is the allele frequency of the SNP in the population. p can be estimated using the allele frequency of the SNP in an ascertained sample by deriving the 2×2 contingency table from the odds ratio e^β , allele frequency in the ascertained sample p_{cc} , and number of cases N_1 and controls N_0 .

Competitive mixture of experts

We consider 14 MR methods, for which instruments can be supplied using two instrument selection strategies, leading to 28 methods in total. In the context of this analysis, each method is considered to be an ‘expert’, taking a set of SNP-exposure and SNP-outcome effect sizes and their standard errors as inputs. Our objective is to select the expert most likely to be correct for a specific MR analysis.

Mixture of experts

The mixture of experts (MoE) method is a machine learning approach which seeks to divide a parameter space into subdomains, such that a particular expert is used primarily for problems that reside in a subdomain most suited to that expert. In this case our objective is to identify characteristics of the SNP-exposure and SNP-outcome associations for which one specific MR method is most likely to yield highest statistical power for non-null associations, and lowest false discovery rates for null associations.

Training and testing simulations

The MoE is trained using datasets generated from simulations. A *dataset* is the minimum data required to perform 2SMR analysis - four columns comprising the SNP-exposure and SNP-outcome effects and standard errors, and rows corresponding to each SNP that is used as an instrument for the exposure. This *dataset* can be fed into any of the 28 experts to obtain MR causal effects. The simulations used to generate these datasets seek to cover a range of pleiotropic scenarios, including where some proportion of SNPs exhibit directional or balanced horizontal pleiotropy, or where SNPs influencing confounding variables.

We simulate two individual level datasets for which there are N_x and N_y samples, and M SNPs, where each SNP has effect allele frequency of $p_m \sim U(0.05, 0.95)$. These datasets are used to obtain the SNP effects for the exposure trait x and the outcome trait y , respectively, using the following sampling criteria:

$$\begin{aligned} N_x &= \{20000, \dots, 500000\} \\ N_y &= \{20000, \dots, 500000\} \\ K &= \{0, \dots, 10\} \\ M_x &= \{1, \dots, 200\} \\ M_y &= \{1, \dots, 200\} \\ M_{u_k} &= \{5, \dots, 30\} \end{aligned}$$

The $M = M_x + M_y + \sum M_{u_k}$ SNPs can influence x directly, y directly, or some number of confounders u_k directly. Phenotypes for x and y are constructed using

$$x = \sum_i^{M_x} \beta_{gx,x,i} g_{x,i} + \sum_j^{M_y} \beta_{gy,x,j} g_{y,j} + \sum_k^K \beta_{ux,k} u_k + e_x$$

where $\beta_{gx,x}$ is the vector of effects of each of the M_x SNPs that influence x primarily, $\beta_{gy,x}$ is the vector of effects for the M_y SNPs on x , where the M_y SNPs influence y primarily but exhibit horizontal pleiotropic effects on x . We allow some proportion of these effects to be 0. β_{ux} is the vector of effects of each of the K confounders on x . Each u_k variable is constructed using

$$u = \sum_l^{M_u} \beta_{gu,l} g_l + e_l$$

and finally y is constructed using

$$y = \beta_{x,y} x + \sum_i^{M_y} \beta_{gy,y,i} g_{y,i} + \sum_j^{M_x} \beta_{gx,y,j} g_{x,j} + \sum_k^K \beta_{uy,k} u_k + e_y$$

where $\beta_{x,y}$ is the causal effect of x on y . We sample the distribution of direct SNP effects using

$$\begin{aligned}
\beta_{gx,x,i} &\sim N(0, \sigma_{gx,x}^2) \\
\sigma_{gx,x,i}^2 &\sim U(0.01, 0.1) \\
\beta_{gy,y,j} &\sim N(0, \sigma_{gy,y}^2) \\
\sigma_{gy,y,j}^2 &\sim U(0.01, 0.1)
\end{aligned}$$

Some proportion $s_x \sim U(0, 1)$ of g_x SNPs and some proportion $s_y \sim U(0, 1)$ of g_y SNPs exhibit horizontal pleiotropy with effects sampled using

$$\begin{aligned}
\beta_{gx,y,i*} &\sim N(\mu_{gx,y}, \sigma_{gx,y}^2) \\
\mu_{gx,y,i*} &\sim U(-0.005, 0.005) \\
\sigma_{gx,y,i*}^2 &\sim U(0.001, 0.01) \\
\beta_{gy,x,j*} &\sim N(\mu_{gy,x}, \sigma_{gy,x}^2) \\
\mu_{gy,x,j*} &\sim U(-0.005, 0.005) \\
\sigma_{gy,x,j*}^2 &\sim U(0.001, 0.01)
\end{aligned}$$

The genetic influences on each of the confounders are sampled using

$$\begin{aligned}
\beta_{gu,u,l} &\sim N(0, \sigma_{gu,u}^2) \\
\sigma_{gu,u,l}^2 &\sim U(0.01, 0.1)
\end{aligned}$$

The influence of each confounder on x and y is obtained using

$$\begin{aligned}
\beta_{u,x} &\sim N(0, \sigma_{u,x}^2) \\
\beta_{u,y} &\sim N(0, \sigma_{u,y}^2)
\end{aligned}$$

Finally, 20% of the simulations have a null effect of $\beta_{x,y} = 0$, while the other remaining 80% have a true effect sampled from

$$\begin{aligned}
\beta_{x,y} &\sim N(0, \sigma_{x,y}^2) \\
\sigma_{x,y}^2 &\sim U(0.001, 0.1)
\end{aligned}$$

For each simulation we used linear regression to estimate the genetic effect of each SNP M on x in sample 1, and each SNP M on y in sample 2. We then perform MR analysis in both directions, retaining SNPs that have $p < 5e - 8$ in sample 1 to perform MR of x on y (the true causal direction for non-null simulations), and retaining SNPs that have $p < 5e - 8$ in sample 2 to perform MR of y on x (the reverse causal direction for non-null simulations). We treat the summary data (effect sizes and standard errors) used for estimating $x \rightarrow y$ the summary data used for estimating $y \rightarrow x$ as two separate datasets. Hence, for each simulation two datasets are generated which are analysed to produce 28 MR estimates each. We performed 100,000 simulations using these parameters, resulting in 200,000 datasets.

Strategy

Figure 2a outlines the general strategy behind the MoE implementation. Briefly, we record 53 metrics about each dataset, hypothesising that we can use these metrics to predict the performance of each expert, given some optimisation criteria. For each method we train random forest decision trees to predict that method's performance using the dataset's metrics.

Having generated random forest decision trees for each of the 28 methods using 133,000 of the simulations, we then applied them to the remaining 67,000 datasets to predict which method would have the highest performance for each of the remaining datasets. Finally we compare the performance of the method selected

by the MoE against all remaining methods. The default settings for the `randomForest` package in R was used to train the models.

Optimisation function

We aim to maximise statistical power for datasets where $\beta_{x,y} \neq 0$ and minimise false discovery rates for datasets where $\beta_{x,y} = 0$. The random forest for a particular method $h(O_{w,d}, \mathbf{z}_d)$ is generated where the training set of input metrics for dataset d is \mathbf{z}_d and the response is

$$O_{w,d} = \begin{cases} 1, & \text{if } \beta_{x,y} \neq 0 \text{ and } p_{m,d} < 0.01 \\ 1, & \text{if } \beta_{x,y} = 0 \text{ and } p_{m,d} > 0.1 \\ 0, & \text{otherwise} \end{cases}$$

Graph database

The set of MR estimates obtained from this analysis are recorded in a Neo4j graph database. Because each association has up to 28 different estimates, for simplicity we distill this down to a single ‘best estimate’ for each association using the following rules:

1. If the number of variants after Steiger filtering is greater than 5 then apply the MoE to obtain the best method
2. If the number of variants after Steiger filtering is less than or equal to 5 but greater than 1 then use the IVW random effects approach on the filtered set of variants
3. If there is 1 variant retained in the Steiger approach then use the Wald ratio on the remaining variant
4. If there are no variants remaining after Steiger filtering then declare no causal association.

For specific hypotheses we strongly recommend that estimates from all sensitivity analyses are scrutinised and reported.

Results

Steiger filtering improves reliability

Mixture of experts method selection improves over any single method

Number of associations using different approaches Which methods were preferentially chosen

A first draft of the causal map of the human phenome

How many associations reach nominal or FDR corrected significance How many bi-directional associations
How many associations could be declared for a causal chain of 2

Discussion

- Limitations of MR that cannot be addressed without biological information
 - Impossible causal associations
 - Temporal effects
 - Risk liability etc
 - Single variants
- Further developments required to improve causal estimates
- The use of causal chains to improve power

References

1. Davey Smith G, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *International Journal of Epidemiology* [Internet]. 2003 Feb;32(1):1–22. Available from: <http://www.ije.oxfordjournals.org/cgi/doi/10.1093/ije/dyg070>
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