Bayesian Mendelian Randomization identifies disease causing proteins via pedigree data, partially observed exposures and correlated instruments

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Abstract

Background

In a study performed on multiplex Multiple Sclerosis (MS) Sardinian families to identify disease causing plasma proteins, application of Mendelian Randomization (MR) methods encounters difficulties due to relatedness of individuals, correlation between finely mapped genotype instrumental variables (IVs) and presence of missing exposures.

Method

We specialize the method of Berzuini et al (2018) to deal with these difficulties. The proposed method allows pedigree structure to enter the specification of the outcome distribution via kinship matrix, and treating missing exposures as additional parameters to be estimated from the data. It also acknowledges possible correlation between instruments by replacing the originally proposed independence prior for IV-specific pleiotropic effect with a g-prior. Based on correlated ($r^2 < 0.2$) IVs, we analysed the data of four candidate MS-causing proteins by using both the independence and the g-prior.

Results

95% credible intervals for causal effect for proteins IL12A and STAT4 lay within the strictly negative real semiaxis, in both analyses, suggesting potential causality. Those instruments whose estimated pleiotropic effect exceeded 85% of total effect on outcome were found to act in trans. Analysis via frequentist MR gave inconsistent results. Replacing the independence with a g-prior led to smaller credible intervals for causal effect.

Conclusions

Bayesian MR may be a good way to study disease causation at a protein level based on family data and moderately correlated instruments.
Key Messages

- Bayesian thinking facilitates elaboration of the basic MR model to deal with challenging dataset, such as pedigree data with partially observed exposures and correlated instruments.
- We incorporate kinship structure in the Bayesian model, in an attempt to reduce the biasing effect of unobserved gene-related confounders.
- Use of g-priors may help dealing with correlated IVs, so as to take advantage from fine-mapping genotyping.
- We deal with missing exposures by treating them as additional parameters to be estimated from the data.
- Our Bayesian analysis consistently points to proteins IL12A and STAT4 as potential causes of MS. Frequentist analyses of the same data yield more confident, but less consistent, results.

Keywords: causal diagrams; Hamiltonian Markov chain Monte Carlo; correlated instruments; missing data; pedigree data; pleiotropy
Introduction

Genome-wide association studies (GWASs) have found a wealth of genetic associations with diseases and traits. The functional meaning of these associations remains largely unknown. This may be due to: (i) the associated variant being in Linkage Disequilibrium (LD) with, but distinct from, the causal one, (ii) the partner causal gene of the variant not necessarily being the nearest to it and, (iii) lack of longitudinal assessments of gene function.

A useful tool in this elucidation effort is the class of methods under the Mendelian Randomization (MR) heading. The bare bones of the MR idea are that for an exposure ($X$) to be a causal influence on an outcome ($Y$), we, under certain assumptions, expect there to be a genetic variant ($Z$) that modulates $X$ to likewise affect $Y$. Information about $Z$ can then be used as an instrument to assess the causal effect of $X$ on $Y$, despite confounding; in which case $Z$ is called an instrumental variable (IV) for the effect of $X$ on $Y$.

Our illustrative example involves data from Multiple Sclerosis (MS) multiplex families, which we use to assess the possible disease-causing effect of four selected proteins, selected in the light of results from a GWAS by the International Multiple Sclerosis Genetic Consortium (IMSGC). Thus, in our analysis, $X$ represents the plasma level of a protein of interest, and $Y$ is a 0-1 indicator of disease status, whereas $Z$ is the instrumental information provided by Immunochip data. We extend the Bayesian MR method of Berzuini et al. to deal with pedigree data, by allowing an estimate of the kinship matrix to enter the specification of the multivariate distribution of $Y$, so as to incorporate pedigree-induced relatedness in the model.

Moreover, our model enhances the ability of the method by Berzuini et al. to deal with weakly correlated IVs, for a more informative analysis of the finely mapped Immunochip data. This is done by acknowledging correlation among IVs in the prior distribution of the pleiotropic effects, specifically by using a $g$-prior.
We jointly analyse two samples of individuals. One of them, which we shall referred to as Sample 1, contains individuals with a complete set of values for $X$, $Y$, and $Z$. The remaining individuals, which we label as Sample 2, have no measured value for $X$. From a Bayesian point of view, the distinction between samples is artificial, as we may just think in terms of a single sample with some missing exposures, to be treated as additional parameters to be estimated from the data, as is customary in Bayesian analysis.

**Methods**

**Sample description**

MS patients were ascertained through the case register established in 1995 in the province of Nuoro, Sardinia, Italy. Cases were diagnosed according to Poser’s criteria\(^\text{10}\). Twenty extended MS multiplex pedigrees were selected for the analysis for a total of $N=936$ individuals (98 cases and 838 unaffected relatives) which were used to calculate the kinship matrix. Two subsets of the $N$ individuals were analysed: Sample 1 (consisting of $N_1$ individuals) with a completely observed $X$, $Y$ and $Z$, and Sample 2 (consisting of $N_2$ individuals) with observed $Y$ and $Z$, and unobserved $X$, with $N=N_1+N_2$.

**Genotyping data**

Genotyping data were obtained by using Immunochip Illumina Infinium HD custom array, designed for fine mapping of 184 established autoimmune loci\(^\text{6}\). The quality control-filtered dataset included 127,134 Single Nucleotide Polymorphisms (SNPs)\(^\text{11}\). Our analysis was performed on a reduced set of 19,121 weakly correlated SNPs ($r^2 < 0.20$ within a window size of 100 Kb) obtained via PLINK’s \texttt{indep-pairwise} command\(^\text{12}\).
**Protein profiling**

Plasma profiles were analysed by using a bead-based antibody array format, consisting of polyclonal Human Protein Atlas\textsuperscript{13} antibodies immobilized onto microspheres in suspension\textsuperscript{14,15} (see Supplementary Material for details).

**Selection of four candidate proteins**

Four candidate proteins (EVI5, IL12A, IRF8 and STAT4) were selected a priori on the basis of Genome-Wide Significant (GWS) association between MS and genetic variants located within (e.g. exonic, intronic, in the UTR) or in the proximity of the protein-coding genes (e.g. downstream, intergenic)\textsuperscript{6}. Odds ratios and \(p\)-values for the strongest associations are reported for each gene in the Supplementary Material.

**Selection of instruments**

On Sample 1, we fitted a separate linear mixed-effects regression model of dependence of each candidate plasma protein level on each SNPs by allowing relatedness between individuals to be accounted for in the model through the kinship matrix, and by adjusting for sex and disease status\textsuperscript{16} (\texttt{lme4} R function). Genetic variants with a significant marginal association (\(p<5\times 10^{-3}\)) and \(r^2<0.20\) were selected to act as IVs in our analysis. The liberal \(p<5\times 10^{-3}\) threshold is justified by the fine genotyping of candidate gene regions and recent arguments\textsuperscript{17,18} in favour of using sub-GWS loci to strengthen biologically interesting signals.

**Bayesian Mendelian Randomization**

Now suppose we have two samples: Sample 1 containing individuals with observed \(X\), \(Y\) and \(Z\), and Sample 2 with observed \(Y\) and \(Z\), but unobserved \(X\) (See Supplementary Table SM1 for details).
In order to robustify the logistic regression part of the model, we transform $X$ and $Z$ to have mean 0 and standard deviation 0.5, as suggested in Gelman et al.\textsuperscript{19}. Let the symbols $X$, $Y$, $U$ hereafter denote $N \times 1$ vectors of individual-specific values, with $N$ denoting total number of individuals. Let the symbol $Z$ hereafter denote the $N \times J$ matrix of values of $J$ instruments in $N$ individuals. Let $\Sigma$ denote the given $N \times N$ kinship matrix. Let the symbol $p$ denote the $N \times 1$ vector containing each individual’s prospective probability of disease. We adopt the following extended version of the model by Berzuini et al.\textsuperscript{7}:

\begin{align*}
U & \sim \text{Normal}(0,1) \\
Y | X, Z, U & \sim \text{Bernoulli}(p) \\
X | Z, U & \sim \text{Normal}(\mu_x, \sigma_x^2) \\
\text{logit}(p) & \sim \text{MVNormal}(\mu_y, \Sigma) \\
\mu_y & = Z\beta + X\theta + U\delta_y \\
\mu_x & = Z\alpha + U\delta_x
\end{align*}

where the symbol $\sim$ is a shorthand for “is distributed as”, $\text{Normal}(w,q)$ and $\text{MVNormal}(w,q)$ respectively denote normal and multivariate normal distribution with mean $w$ and (co)variance (matrix) $q$, $\alpha \equiv (\alpha_1, \ldots, \alpha_J)$ is the vector of i-e associations; $\beta \equiv (\beta_1, \ldots, \beta_J)$ the vector of pleiotropic effects, and $\theta$ is the causal effect of interest, i.e., the change in log-odds of disease corresponding to an interventional unit change in $X$. Let the total effect for the generic, $j$th, instrument be defined as $\gamma_j = \alpha_j\theta + \beta_j$, where $\alpha_j\theta$ is the indirect effect and $\beta_j$ the direct effect of the IV, on a log-odds scale.

A graphical representation of the model, which uses the Winbugs "plate" formalism\textsuperscript{20}, is given in Figure 1.

We complete the Bayesian model with the following prior specifications:
• $\alpha_j$ follows a $Normal(0, \sigma^2_\alpha)$ prior, where the unknown quantity $\sigma_\alpha$ is a priori
  distributed as a half-Cauchy distribution on the positive reals with location 0 and scale 1,
• the individual-specific values of $U$ and $\theta$ follow independent standard normal priors,
• the parameters $\delta_x, \delta_y$ are assigned $Normal(0,0.25)$ priors,
• the vector $\beta$ is a priori $MVNormal(0, \sigma^2\tau A)$ with given matrix $A=(Z^TZ)^{-1}$, and with
  parameters $\sigma$ and $\tau$ discussed in the next section.

A discussion of the identifiability properties of this model in the special case where $\Sigma$ is
diagonal and $\beta$ subject to an independence shrinkage prior is given in Berzuini et al.\textsuperscript{7}.

The vector $\beta$ and the parameter $\theta$ are also unidentifiable from the likelihood, but they have
proper posteriors under the above model when $\beta$ is subject to a shrinkage prior, this latter
translating the biologically plausible assumption that some instruments exert no pleiotropic
effect on the outcome. In Berzuini et.al.\textsuperscript{7} the shrinkage is obtained by a horseshoe prior that
takes the $\beta$ to be independent. In contrast, our model explicitly acknowledges IV correlation
by taking $\beta$ to follow the $g$-prior\textsuperscript{21} discussed in the next section. A further novelty in our
proposal is taking the vector of logit probabilities of disease to have a multivariate normal
distribution that accounts for the pedigree-induced individual-individual correlations implicit
in the kinship matrix, $\Sigma$.

\textbf{Modelling instrument-instrument correlations via $g$-priors}

In many applications, it is customary to assume that an unknown subset of pleiotropic effects
has value zero. This (often plausible) assumption is sufficient under the above model to obtain
a proper posterior distribution for the parameter of inferential interest, $\theta$. In Berzuini et.al.\textsuperscript{7},
these considerations justify the use of an independence shrinkage prior for $\beta$. But in those
situations where we have an ensemble of weakly correlated IVs, a more appropriate choice of
a prior for $\beta$ is a prior that captures the idea of a possible correlation among its component. We here propose the use of a g-prior for $\beta$. Following Liquet et al.\textsuperscript{21}, we specify the g-prior as:

$$\beta \sim \text{MVNormal}(0, \sigma^2 H_f),$$

where $\sigma^2$ follows a priori an inverse Gamma distribution with chosen hyperparameters $a, b$, although in our final model we fix $\sigma^2 = 1$, as suggested in Marin and Robert\textsuperscript{23}. $H_f$ is defined as:

$$H_f = \tau A$$

where the scaling parameter $\tau$, that governs the selection of variables, is assigned an inverse Gamma prior with hyperparameters $1$ and $N_1$. By imposing the $\beta$ coefficients a correlation structure that mirrors the likelihood, the g-prior, acts as a shrinkage device, it discourages simultaneous inclusion in the model of instruments that are positively correlated\textsuperscript{21}.

\textit{Computational method}

Our Bayesian analyses were performed by using the Hamiltonian Markov chain Monte Carlo (MCMC) facilities offered by the STAN language\textsuperscript{24} (http://mc-stan.org/) via the Rstan R interface\textsuperscript{25}. Relevant STAN code is given in the Supplementary Material. We checked the goodness of fit of each model by comparing outcome distribution statistics (posterior mean and standard deviation) from datasets simulated from the entertained model with the corresponding observed statistics in the spirit of Gelman’s predictive checks\textsuperscript{26}.

\textit{Frequentist Mendelian Randomization}

For purposes of comparison, we re-analysed the data by using the following frequentist MR methods, without altering the set of IVs: Inverse-Variance Weighted estimator (IVW)\textsuperscript{27,28}, MR-Egger Regression estimator (MR-ER)\textsuperscript{29,30} and Weighted Median Estimator (WME)\textsuperscript{28,31}.
These methods, as provided by the MendelianRandomization R package\(^{32}\), are able to work from summary statistics, and assume the instruments to be independent. Summary statistics from the regressions of each candidate protein level on each IVs were obtained from Sample 1. Summary statistics from the regressions of the MS indicator on selected IVs, in the form of estimated log-odds ratios, were obtained from a previous study of ours\(^{11}\).

**Results**

*Selection of IVs*

Table 1 shows for each protein the total number of selected IVs (see Supplementary Table SM2 for details).

*Results of Bayesian Mendelian Randomization*

Table 2 reports the results obtained by using the Bayesian method of Berzuini et al.\(^ {7}\) (Analysis 1) and those by using the same method with g-priors (Analysis 2).

For each protein and for each analysis, the posterior mean for the causal effect, $\hat{\theta}$, its corresponding 95% posterior Credible Interval (CI) and its standard deviation (sd) are reported. The two analyses agree on the causal effect $\theta$ of each candidate protein having a negative point estimate and on the posterior distribution for the causal effects of IL12A and STAT4 being well separated from the null. According to Analysis 2, $\hat{\theta}$ is significantly different from the null also for EVI5 and IRF8 proteins. Recall that $\theta$ represents the causal effect on MS on a log-odds ratio scale. Posterior estimates did not vary dramatically across the two analyses, and convergence of the Markov chain was satisfactory in both of them.

Figure 2 shows the estimated pleiotropic effects ($\beta$s) for a subset of the instrumental SNPs, as obtained from Analysis 2. Also displayed are the corresponding 95% CIs. Along the vertical right border of the figure, the $\beta$s are expressed as percentages of their corresponding total
effects, on a log-odds of MS scale, according to Analysis 2. The SNPs selected for the plot have this percentage equal or greater than 0.85.

In Analysis 1 all CIs for $\beta$s turned out to cover the null value.

Supplementary Figures SM1-SM5 show similar plots for each protein, for both Analysis 1 and 2. Posterior predictive check diagnostics do not reveal evidence of model misfit (see Supplementary Figures SM6-SM9).

Results from frequentist Mendelian Randomization

Table 3 reports the results of the frequentist MR analysis for the four candidate proteins. According to this analysis, IL12A exhibits a significant and consistent causal effect on MS according to both IVW ($\hat{\theta}=-0.18, p<0.0001$) and WME ($\hat{\theta}=-0.1116, p=0.012$) but not MR-ER ($\hat{\theta}=-0.227, p=0.108; \hat{\theta}_0=-0.024, 95\% \text{ CI}: -0.112 \text{ to } 0.160, p=0.729$). IRF8 exhibits a significant and consistent causal effect on MS only according to IVW ($\hat{\theta}=-0.107, p=0.004$). EVI5 exhibits a significant and consistent causal effect on MS only according to MR-ER ($\hat{\theta}=0.328, p=0.02; \hat{\theta}_0=-0.161, 95\% \text{ CI}: -0.289 \text{ to } -0.033, p=0.013$).

Discussion

A recently proposed Bayesian framework for MR\textsuperscript{7} lends itself to a number of (still largely unexplored) developments. Two of them are enabling MR to deal with pedigrees and to optimally use large collections of correlated IVs. And, finally, there is a demand for formulations of MR which enable this class of methods to work in a wider spectrum of areas of application.

This paper addresses and combines the three mentioned issues.

MR methods have been frequently applied to the study of high-level exposures, such as obesity\textsuperscript{33,34}. Our illustrative study turns the attention to disease-causing agents that operate at
an earlier stage of the causal chain: the proteins. By focusing on proteins, we are professing interest in exposures that are (in some sense) closer to the genetic instruments. We would hardly be able to judge how successful MR can be in this application context without experimenting on real data, as we do in this paper. Another idea in this paper, which, to the best of our knowledge, is new in MR literature, is to apply the method to pedigrees. Sample individuals belonging to the same family tend to display a lower degree of genetic heterogeneity, especially if they are drawn from a founder and isolated population as Sardinian, as in our study. One reason being that, in this case, the individuals also share a large portion of environmental variability. The low heterogeneity facilitates detection of causality at the level of proteins. Another reason for our interest in family data is that they reveal, to a larger extent than other sources, the underlying kinship structure of the studied individuals. By incorporating this information in the model, as we have done, we (to some degree) reduce the biasing effect of unobserved gene-related confounders. Researchers aware of the vulnerability of MR to unobserved confounding and to untestable violations of the assumption of instrument-confounder independence cannot underappreciate that feature.

The collection of variants that we chose to act as IVs in our analyses can be subdivided in two groups, according as that variant is located in the encoding gene, in which case we say it is acting in cis, or in the distal region of the gene, in which case we say it is acting in trans. Figure 2 displays point and interval estimates for the instrument-specific pleiotropic effects, for a protein of interest. Interestingly, we found that the majority of the a posteriori important pleiotropic effects (those that exceed 85% of the corresponding total effect on the outcome) act in trans. This finding agrees with the general expectation that trans-acting variants are more likely to exert large pleiotropic effects. Results of our analysis point to one of the four candidate proteins, IL12A, as a potential cause of MS. IL12A is an immunomodulatory cytokine that can act as a growth factor for activated T and natural killer (NK) cells, enhance
the lytic activity of NK/lymphokine-activated Killer cells, and stimulate the production of IFN-gamma by resting PBMC\textsuperscript{35}. This protein is highly involved in autoimmune system activation and its involvement in MS pathogenesis and development is becoming clear\textsuperscript{36}. Results of our analysis confirm the tendency of the Bayesian approach to yield wider CIs for the causal effect, compared to the frequentist approach. This may be due to the Bayesian method accounting for sources of uncertainty at all levels of the model, including the uncertainty in the instrument-exposure associations, and agrees with what previous authors found\textsuperscript{7}. The same authors performed simulations showing that the more conservative intervals obtained from a Bayesian analysis correspond to better power and coverage properties of the estimator. Our results also show a marked inconsistency of the frequentist estimates across different algorithms. Our comparison of Bayesian analyses that use different priors for the pleiotropic effects (Analyses 1 and 2) confirm our expectation, that the $g$-prior, thanks to its acknowledging of the correlation, induced among the instruments by the LD, yields narrower CIs.

Finally, this paper offers a convincing case for adopting Bayesian thinking in MR. In this paper we elaborate the basic MR model in various ways, one of these being by incorporating family structure into the model. Elaborations of this kind may be extremely hard within a frequentist approach to estimation. But they may be an easy task for a Bayesian. Graphical representations such as Figure 1 have an important role in the generalization of a Bayesian model and in the identification of the set of (causal and conditional independence) assumptions that make the generalization possible. At the level of the graph, elaborating the model can be as easy as adding a node to it, as we have, for example, done by adding node $\Sigma$ to represent kinship information. Then, by mapping our biological knowledge onto the graph, we check whether the new required assumptions are plausible and, in case of a positive answer, we proceed by calculating the estimates of interest, which involves the graph as a
support structure for the necessary MCMC calculations. A final, important, benefit of Bayesian analysis is its natural ability to combine evidence from different samples characterized by observation of different subsets of \((X, Y)\). From a Bayesian point of view, all the individuals are, under due assumptions, regarded as belonging to the same sample, although in some individuals, the values of either \(X\) or \(Y\) are missing, in which case they are simply treated as additional parameters to be estimated from the data.

**Funding**

This work was supported by Fondazione Italiana Sclerosi Multipla [grant number 2009//R//2] and Fondazione Cariplo [grant number 2009-2528].

**Conflict of interest.** None declared
**Table 1:** Total number of available IVs for each candidate protein.

| Protein (Antibody ID) | Total Number of IVs |
|-----------------------|---------------------|
| IL12A (hpa001886)    | 76                  |
| IRF8 (hpa002531)     | 116                 |
| EVIS (hpa027339)     | 106                 |
| STAT4 (hpa00186)     | 102                 |

IV: Instrumental Variable

**Table 2:** Results of the Bayesian MR analysis for the candidate proteins.

| PROTEIN (Antibody ID) | Analysis 1 | Analysis 2 |
|-----------------------|------------|------------|
|                       | \( \hat{\theta} \) | 95% C.I. | sd | \( \hat{\theta} \) | 95% C.I. | sd |
| IL12A (hpa001886)    | -0.202    | [-0.418, -0.091] | 0.078 | -0.228    | [-0.318, -0.141] | 0.046 |
| IRF8 (hpa002531)     | -0.117    | [-0.304, 0.064] | 0.092 | -0.254    | [-0.361, -0.145] | 0.056 |
| EVIS (hpa027339)     | -0.223    | [-0.58, 0.124] | 0.176 | -0.248    | [-0.38, -0.128] | 0.062 |
| STAT4 (hpa00186)     | -0.247    | [-0.462, -0.06] | 0.103 | -0.226    | [-0.326, -0.124] | 0.051 |

\( \hat{\theta} \): posterior mean of the causal effect of interest; C.I.: Credible Interval; sd: posterior standard deviation

Analysis 1: Bayesian MR as in Berzuini et al

Analysis 2: Bayesian MR with \( g \)-priors

**Table 3:** Results of frequentist MR analysis on the selected four candidate proteins.

| PROTEIN (Antibody ID) | IVW |              |              | MR-ER |              |              | WME |              |
|-----------------------|-----|--------------|--------------|-------|--------------|--------------|-----|--------------|
|                       | \( \hat{\theta} \) | 95% CI | P-value | \( \hat{\theta} \) | 95% CI | P-value | \( \hat{\theta} \) | 95% CI | P-value |
| IL12A (hpa001886)    | -0.18 | [-0.226, -0.095] | <0.0001 | -0.227 | [-0.503, 0.05] | 0.108 | -0.166 | [-0.296, -0.037] | 0.012 |
| IRF8 (hpa002531)     | -0.107 | [-0.179, -0.034] | 0.004 | -0.026 | [-0.265, 0.213] | 0.831 | -0.052 | [-0.156, 0.051] | 0.322 |
| EVIS (hpa027339)     | -0.007 | [-0.084, 0.070] | 0.859 | 0.328 | [0.052, 0.604] | 0.020 | 0.007 | [-0.103, 0.118] | 0.899 |
| STAT4 (hpa00186)     | -0.039 | [-0.135, 0.056] | 0.417 | 0.268 | [-0.031, 0.567] | 0.079 | -0.057 | [-0.172, 0.059] | 0.338 |

\( \hat{\theta} \): causal effect estimate, CI: Confidence Interval; IVW: Inverse Variance Weighted; MR-ER: MR-Egger Regression; WME: Weighted Median Estimator
**Figure Legends**

**Figure 1**: Graphical representation of Model (1)-(6). Rectangular nodes represent fixed variables. Circular nodes represent random variables. Individual-specific variables are represented as nodes within the plate, while model parameters are located outside the plate. Graph should be interpreted as a conditional independence directed acyclic graph according to\textsuperscript{22}. In this figure the parameters that enter the distribution specification of an unobservable variable are represented as direct parents of that variable.
Figure 2: A posteriori important pleiotropic effects ($\beta$) estimate and corresponding 95% Credible Intervals (CIs) for IL12A, according to Analysis 2. Along the left vertical axis are IV labels, while along the right vertical border of the figure, the pleiotropic effects are expressed as percentage of their corresponding total effect greater or equal to 0.85.
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