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SLOPE-HUNTER: A ROBUST METHOD FOR INDEX-EVENT BIAS CORRECTION IN GENOME-WIDE ASSOCIATION STUDIES OF SUBSEQUENT TRAITS

A PREPRINT

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January 31, 2020

ABSTRACT

Background

Studying genetic associations with prognosis (e.g. survival, disability, subsequent disease events) is problematic due to selection bias - also termed index event bias or collider bias - whereby selection on disease status can induce associations between causes of incidence with prognosis. A current method for adjusting genetic associations for this bias assumes there is no genetic correlation between incidence and prognosis, which may not be a plausible assumption.

Methods

We propose an alternative, the 'Slope-Hunter' approach, which is unbiased even when there is genetic correlation between incidence and prognosis. Our approach has two stages. First, we use cluster-based techniques to identify: variants affecting neither incidence nor prognosis (these should not suffer bias

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and only a random sub-sample of them are retained in the analysis); variants affecting prognosis only (excluded from the analysis). Second, we fit a cluster-based model to identify the class of variants only affecting incidence, and use this class to estimate the adjustment factor.

Results

Simulation studies showed that the Slope-Hunter method reduces type-1 error by between 49%-85%, increases power by 1%-36%, reduces bias by 17%-47% compared to other methods in the presence of genetic correlation and performs as well as previous methods when there is no genetic correlation. Slope-Hunter and the previous methods perform less well as the proportion of variation in incidence explained by genetic variants affecting only incidence decreases.

Conclusions

The key assumption of Slope-Hunter is that the contribution of the set of genetic variants affecting incidence only to the heritability of incidence is at least as large as the contribution of those affecting both incidence and prognosis. When this assumption holds, our approach is unbiased in the presence of genetic correlation between incidence and progression, and performs no worse than alternative approaches even when there is no correlation. Bias-adjusting methods should be used to carry out causal analyses when conditioning on incidence.

***K*eywords** GWAS · Case-only studies · Collider bias · Index event bias · Cluster-based models

1 Background

There is increasing interest in the use of genome wide association studies (GWAS) not only to investigate risk of disease, but to examine prognosis or outcome of people with the disease [1, 2, 3]. Studies of prognosis, of necessity, can be conducted only in those who have the disease, i.e. conditioning on disease incidence. This leads to a type of selection bias - termed index event bias or collider bias - whereby uncorrelated causes of the disease appear correlated when studying only cases [1, 2, 4]. This means that if there is unmeasured confounding between incidence and prognosis, then any cause of incidence will appear also to cause prognosis. Any cause of both incidence and prognosis will have a biased estimate of its effect on prognosis.

Figures 1 and 2 illustrate that a single nucleotide polymorphism (SNP), G, causing disease trait, I, becomes correlated with the confounder, U, of disease and subsequent trait, P, when conditioning on I. This induces association between G and P via the path $G - U \rightarrow P$ leading to index event bias in the SNP-prognosis association, if the confounding effects are not accounted for. If all causes of incidence were known and could be measured, the selection bias could then be removed, e.g. by using the inverse probability weighting (IPW) approach [5]. But, for IPW to be valid, the weighting model must be correctly specified, and must include all variables that are related to both incidence and to the

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variables in the analysis model (e.g. to prognosis and every genetic variant). However in most studies, these variables are not all known, and not all are measured.

The implications of index event bias have been addressed in several GWAS and MR studies [1, 2]. An example is the 'paradox of glucose-6-phosphate dehydrogenase (G6PD) deficiency' whereby among individuals selected according to their status of severe malarial anaemia (SMA), higher levels of G6PD deficiency appear to protect against cerebral malaria (CM) [6, 7]. A possible explanation is that if an individual with SMA has a high level of G6PD deficiency, they may well have lower levels of other risk factors for SMA. If lower levels of those other factors tend to decrease risk of CM, then the G6PD deficiency may appear to be protective against CM. In the notation of Figure 1, G6PD deficiency plays the role of the SNP G_1 whereas I and P represent SMA and CM respectively. It has been suggested that this apparent protective effect is at least partially due to index event bias (collider bias) [8].

A method for adjusting genetic associations for the index event bias has been proposed whereby estimated residuals from the regression of SNP-prognosis associations on SNP-incidence associations give bias-adjusted effects on prognosis [4]. This method assumed that the direct genetic effects on incidence and prognosis are linearly uncorrelated. But, this assumption may be incompatible with the premise of genetic studies in which shared pathways of incidence and prognosis have been observed for many traits. For example, such shared pathways may be common for psychiatric traits [9], metabolites [10] and phenotypes related to cumulative effects of long-term exposures [11].

We propose a novel method, referred to as 'Slope-Hunter', for adjustment of index event bias in GWAS of progression studies with potentially correlated direct genetic effects on incidence and prognosis. This is achieved by first identifying the class of SNPs which only affect incidence, and using these to obtain an unbiased estimate of the correction factor that is then used to adjust for the bias for all genetic variants. We evaluate the Slope-Hunter method by comparing its type-1 error, power and bias with the previously proposed methods in an extensive simulation study with realistic parameters.

2 Methods

For an individual SNP, it is assumed that a continuous incidence trait I is linear in the coded genotype G, common causes U of incidence and prognosis, and unique causes ε_I of I:

$$I = \beta_{GI}\, G + \beta_{UI}\, U + \varepsilon_I. \tag{1}$$

Moreover, we assume that a continuous prognosis trait P is linear in G, U, and unique causes ε_P of P, with an additional linear effect of I:

$$P = \beta_{GP}\, G + \beta_{UP}\, U + \beta_{IP}\, I + \varepsilon_P. \tag{2}$$

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The effect of our interest is the SNP effect on prognosis, β_{GP}, conditional on incidence I and confounders U. However in practice, we can only estimate the SNP-prognosis association conditional on incidence, denoted by β'_{GP} in equation 3, because all relevant confounders may not be observed.

$$E\,(P\,|G, I\,) = \beta'_{GP}\,G\;+\;\beta'_{IP}\,I, \tag{3}$$

where β'_{GP} is a biased estimate of SNP effect on prognosis (termed 'conditional estimate'), whereas β'_{IP} is a biased estimate of the causal effect of incidence on prognosis. Equations 1 and 2 assumed that both I and P are continuous traits. However, if I and/or P are binary, as in the case for disease traits, it has been shown that the logistic and probit link functions are approximately linear for small effects, as typically is the case for polygenic traits [4]. Therefore, we still consider the linear models presented in equations 1 and 2. If the incidence is a binary disease trait, then conditioning on the incidence, as in equation 3, is equivalent to analysing prognosis among cases only:

$$E\,(P\,|G, I = 1\,) = \beta'_{GP}\,G\;+\;\beta'_{IP}, \tag{4}$$

Dudbridge et. al. (2019) showed that the conditional estimate β'_{GP} can be formulated as the true effect β_{GP} plus a bias that is linear in the SNP effect on incidence β_{GI} [4]. In particular, the conditional estimate can be expressed as follows:

$$\beta'_{GP} = \beta_{GP} + b\,\beta_{GI}, \tag{5}$$

$$b = \frac{-\,\sigma^2_U\;\beta_{UI}\;\beta_{UP}}{\sigma^2_U\;\beta^2_{UI} + \;\sigma^2_{\varepsilon_I}}, \tag{6}$$

where σ^2_U and $\sigma^2_{\varepsilon_I}$ are variances of confounders and residual unique causes of I respectively. Therefore, by regressing the conditional estimates β'_{GP} on β_{GI} for all SNPs, the slope, b, could be estimated using ordinary least squares (OLS), assuming that [4]:

- A_1: The effects of SNPs on incidence are linearly uncorrelated with their direct effects on prognosis (i.e. index coefficient linearly uncorrelated with direct effect, referred to as the 'InCLUDE' assumption).
- A_2: The confounder effects - and hence b - are constant across all SNPs.

The estimated slope, $\hat{b}$, can then be used to obtain bias-adjusted effects on prognosis for each SNP by calculating the residuals of equation 5 as follows:

$$\hat{\beta}_{GP} = \hat{\beta}'_{GP}\;-\;\hat{b}\,\hat{\beta}_{GI}. \tag{7}$$

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If there are shared pathways for both incidence and prognosis whereby the direct effects on prognosis are correlated with effects on incidence, e.g. as shown in Figure 3, then the assumption A_1 can be violated producing bias in $\hat{b}$, and hence not correcting adequately for the index event bias, see Figure 4. Although the non-genetic component of U is constant across all SNPs by definition, assuming it has no interaction with each SNP, the genetic component of U may differ across SNPs leading to violation of assumption A_2. For a single SNP G_1 affecting incidence only (Figure 1), the genetic component of U is the entire shared genetic basis of I and P. Whilst for a single SNP G_2 affecting both incidence and prognosis (Figure 2 and Figure 3), the genetic component of U equals entire shared genetic basis of I and P, minus the component attributed to the SNP under consideration, G_2.

In this paper, we propose a novel approach for adjustment of index event bias in GWAS of progression studies, referred to as'Slope-Hunter'. It firstly identifies variants affecting neither incidence nor prognosis, i.e. the class of G_4 SNPs as shown in Figure 5(b); and variants affecting prognosis only, i.e. the class of G_3 SNPs as shown in Figure 5(a). These classes of variants do not have an effect on incidence, and therefore do not suffer bias. A random sub-sample of G_4 SNPs is retained in the analysis to facilitate pattern identification, and the remaining SNPs of these groups are excluded. Then, the pattern of the class of variants affecting incidence only, G_1, is distinguished and used to obtain an unbiased estimate of the slope b.

The 'Slope-Hunter' Method

Equation 5 can be reformulated according to SNP associations with incidence and prognosis as follows:

$$\beta'_{GP} = \begin{cases} b_1\ \beta_{GI}, & for\ variants\ causing\ incidence\ only\ \ (G_1) \\ \beta_{GP} + b_2\ \beta_{GI}, & for\ variants\ causing\ incidence\ \&\ prognosis\ \ (G_2) \\ \beta_{GP}, & for\ variants\ that\ are\ not\ causing\ incidence\ \ (G_3\ or\ G_4) \end{cases} . \tag{8}$$

For the class of variants only affecting incidence, G_1, assumption A_1 is not violated, since these SNPs have no direct effects on prognosis. In addition, the genetic and non-genetic components of U, and hence the slope b_1 is constant across all elements of the class G_1, i.e. assumption A_2 is satisfied. Therefore, regressing the conditional estimates of SNP-prognosis associations, $\hat{\beta}'_{G_1P}$, on estimates of SNP-incidence associations, β'_{G_1I}, enables us to obtain an unbiased estimate, $\hat{b_1}$, for b_1, see equation 8, that can be utilised as an adjustment factor. Although the unbiased estimate of this adjustment factor is obtained by using the class of variants only affecting incidence, G_1, it can still be used to correct bias for all SNPs by substituting $\hat{b}$ with $\hat{b_1}$ in equation 7, assuming that $b_1 \approx b_2$, which is typically the case for small effects as in GWAS.

For G_2, either the assumption A_1, A_2 or both may not be satisfied, e.g. if there are underlying shared biological pathways for I and P and/or there are major variants accounting for substantial covariation in I and P leading to non-constant genetic component of U. In such cases, the slope estimated using the G_2 class of variants, $\hat{b_2}$, might be biased. Figure 2 shows the DAG for a SNP G_2 that has a direct effect on both incidence and prognosis, whereby the

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estimated SNP-prognosis association conditional on incidence, β'_{G_2P}, is due to the collider bias in addition to the SNP's direct effect on prognosis. In such a case, the ratios of $\beta'_{G_2P}/\beta_{G_2I}$ are not constant across all elements of the set of G_2 SNPs.

For G_3 and G_4, the estimated direct effects on prognosis do not suffer from the index event bias, since conditioning on I does not induce association between U and G unless the genetic variant G causes the incidence, see Figure 5.

The Slope-Hunter method utilises cluster-based models [12, 13, 14] to identify the class of G_1 and then adjust for the index event bias. The pseudo code of the 'Slope-Hunter' approach is presented in Algorithm 1 and a graphical illustration of the phases of our approach is presented in Figure 6.

Identification of variants affecting incidence only

Our proposal requires summary-level GWAS statistics and their standard errors for an incidence trait I, $\hat{\beta}_{GI}$ and s_{GI}; and a prognosis trait P conditional on I, $\hat{\beta}'_{GP}$ and s'_{GP}. These inputs are obtained from GWAS of incidence and prognosis conditional on incidence. Other inputs provided by the user are an ordered set Λ of k proportions representing sub-sample sizes λ_j, $j = 1, \ldots, k$ of the class G_4 that are retained in the analysis; a p-value threshold η for identifying SNPs with no effect on incidence, the class G_3; and a tolerance distance scalar δ used for validating the cluster solutions. Our developed algorithm uses default values for these parameters, $\Lambda = \{3\%, 4\%, \ldots, 10\%\}$, $\eta = 0.1$ and $\delta = 1$, that performed the best in our simulation studies. The Slope-Hunter approach produces the bias-adjusted estimates of SNP-prognosis associations $\hat{\beta}_{GP}$ and their estimated standard errors s_{GP}.

Since the adjustment factor, b_1, should be estimated using a set of independent SNPs, GWAS are first pruned by linkage disequilibrium (LD). The first step of our approach, presented in Algorithm 1, uses the pruned GWAS statistics for the incidence I to obtain p-values for SNP-incidence associations, p_{GI} (line 1). Then, we fit a bivariate normal mixture model with two components to estimate probabilities, and to assign memberships, of the cluster G_4. Each mixture component is specified as a two-dimensional ellipse with varying geometric features: area, denoted by ζ (i.e. $\zeta_4 \neq \zeta'_4$); shape, denoted by ϑ (i.e. $\vartheta_4 \neq \vartheta'_4$); orientation, denoted by θ (i.e. $\theta_4 \neq \theta'_4$), between the class G_4 and its complementary cluster G'_4 (line 2). The geometric features of each cluster are determined by its covariance matrix that can be decomposed to the form of $\zeta_k\theta_k\vartheta_k\theta_k$ for a cluster k, where ζ_k is a scaler controlling the cluster area, ϑ_k is a 2×2 diagonal matrix whose determinant equals 1 characterising its shape, and θ_k is a 2×2 orthogonal matrix characterising the orientation of corresponding ellipsoid [15, 16]. Figure 7 illustrates the geometric features of clusters identified by the Slope-Hunter method using the data simulated for Figure 4. Since SNPs of the class G_4 have effects on neither incidence nor prognosis by definition, then their corresponding pairs of association coefficients β_{G_4I} and β'_{G_4P} should have no structural function with respect to neither incidence nor prognosis. Hence their estimated values are expected to scatter around the origin in a noisy form with a high probability concentration, since the majority of GWAS variants are expected to belong to this class, see Figure 6(b). The G_4 class is then identified as the cluster that contains the point with the smallest Euclidean distance from the origin.

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Exclusion of all SNPs assigned to the class G_4 may discontinue the dimensional space leading to poor or invalid clustering of the remaining data. Therefore, we retain a fraction λ of G_4 SNPs to facilitate pattern recognition of the class G_1. The selection of this sub-sample is performed randomly using a weighted score defined as a modified form of the Euclidean distance from the origin, w_g, for each SNP $g \in G_4$ (lines 3-5) . This implies that data points with larger distance from the origin, with higher weights given to the prognosis dimension, are more likely to be retained in the analysis. The weights are then normalised to lie within $[0, 1]$ (line 6).

We define $\mathbb{C}$ as a set of the adjustment factor candidates. This is initialised as an empty set (line 7) that will be iteratively updated by assigning a candidate adjustment factor $\hat{b}_j$ obtained by fitting a clustering solution f_j using a different sub-sample size $\lambda_j \in \Lambda$, at each iteration, $j = 1, \ldots, k$. For each given proportion λ_j, the following steps are performed:

1. A sub-sample of SNPs, ℓ_j, of size λ_j is randomly selected from the class G_4 using the vector of weights ω_{G_4} whose individual element for a single SNP g is ω_g (line 9).

2. The set of SNPs, G^*, retained in the analysis is defined as the selected sample ℓ_j in addition to all SNPs $g \in G_4'$ whose p-values $P_{gI} < \eta$ (line 10). The latter procedure is designed to exclude SNPs that have no effects on I among the cluster G_4' (i.e. it excludes the G_3 group).

3. SNPs of the G_1 class should differ from the G_2 class in terms of their patterns of estimated values around the true slope b_1, because the G_1 SNPs satisfy a proportional relationship between β'_{GP} and β_{GI}, whilst SNPs of the G_2 class deviate from such a proportional form with magnitudes dependent on true size of effect on prognosis for each SNP, β_{GP}, see equation 8. The data points of G_1 and G_2 sets can then be treated as observations generated by two unknown distinct bivariate normal distributions. The Slope-Hunter method fits a cluster-based model (line 11) using the expectation–maximization (EM) algorithm to identify the underlying distributions and to estimate probability of each SNP belonging to the G_1 class. For the set of SNPs G^*, we fit a bivariate normal mixture model with two components, G_1 and G_2 that are ellipsoidal with varying orientations ($\theta_1 \neq \theta_2$), representing varying slopes for G_1 and G_2, but possibly with equal areas and shapes. This is achieved by employing the model with the minimum Bayesian information criterion (BIC) among models with: equal areas and shapes ($\zeta_1 = \zeta_2, \vartheta_1 = \vartheta_2$); varying areas and equal shapes ($\zeta_1 \neq \zeta_2, \vartheta_1 = \vartheta_2$); equal areas and varying shapes $\zeta_1 = \zeta_2, \vartheta_1 \neq \vartheta_2$; varying areas and shapes $\zeta_1 \neq \zeta_2, \vartheta_1 \neq \vartheta_2$.

4. We define $I\left(d_{\mu_1,\mu_2} \leq \delta \,.\, min\left(s_{1I}, s_{1P}, s_{2I}, s_{2P}\right)\right)$ as an indicator that sets to 1 if $d_{\mu_1,\mu_2} \leq \delta \,.\, min\left(s_{1I}, s_{1P}, s_{2I}, s_{2P}\right)$, otherwise it sets to zero (line 12); where d_{μ_1,μ_2} is the Euclidean distance between the means μ_1 and μ_2 of the identified clusters G_1 and G_2, the $s_{1I}, s_{1P}, s_{2I}, s_{2P}$ represent standard deviations of the clusters G_1 and G_2 respectively on each dimension I and P. The scalar, δ is set to scale the minimum standard deviation of the clusters. For example, if $\delta = 1$ (the default), then the obtained cluster solution will be considered as a candidate for bias adjustment only if the distance between means of its components is not

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larger than the minimum standard deviation s_{ij}, $i = 1, 2$, $j = I, P$, i.e. the value of parameter δ allows users to control how to trim the clustering solution candidates.

5. Since the class G_1 is assumed to be scattered around its slope with less variation than the other class G_2, then the Class G_1 is identified as the one producing the minimum standard error of the slope b_j when regressing SNP-incidence on SNP-prognosis associations. If the model f_j produces a valid clustering candidate, as indicated by line 12, then a linear regression model M_j regressing associations with incidence, $\hat{\beta}_{G_1 I}$, on association with prognosis $\hat{\beta}'_{G_1 P}$ for SNPs in the class G_1 is fitted (line 13).
6. The set $\mathbb{C}$ is then updated by adding the element representing the estimated slope $\hat{b}_j$ from the model M_j (line 14).

The optimal adjustment factor, $\hat{b}_1$, is then identified as the slope $\hat{b}_j \in \mathbb{C}$ with the minimum standard error (line 17). Since analyses are conducted on finite sample estimates $\hat{\beta}_{GI}$ and $\hat{\beta}'_{GP}$, the regression may yield an estimate $\hat{b}_j$ that is biased towards zero. Therefore, we have adjusted for such a regression dilution using the Hedges-Olkin estimator [4]. The bias-adjusted estimates of effects on prognosis and their standard errors for all SNPs $g \in G$ can then be obtained using the optimal adjustment factor $\hat{b}_1$ after regression dilution correction (lines 19, 20).

All procedures described in this manuscript have been implemented into an open source R package named 'Slope-Hunter' that would be available from **https://github.com/Osmahmoud/Slope-Hunter**

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Algorithm 1 Slope-Hunter: Adjustment for index event bias in GWAS of subsequent events

Inputs: $\hat{\beta}_{GI}$, $\hat{\beta}'_{GP}$, their standard errors s_{GI} and s'_{GP}, an ordered set Λ of k percentages for sub-sample sizes λ_j, $j = 1, \ldots, k$, p-value threshold η, a tolerance distance scalar δ.
Outputs: $\hat{\beta}_{GP}$, s_{GP}. {Adjusted estimates of SNP-prognosis associations and their standard errors}

$p_{GI} = 2\left[1 - p\left(Z \leq \frac{\hat{\beta}_{GI}}{s_{GI}}\right)\right]$ {p-values for SNP-incidence associations, where $Z \sim N(0,1)$}

Fit a bivariate mixture model with two-components to generate model-based cluster of G_4 using the EM algorithm as follows:

$$f\left(\hat{\beta}_{GI}, \hat{\beta}'_{GP} \,|\pi_4\right) = \pi_4 . G_4\left(\hat{\beta}_{GI}, \hat{\beta}'_{GP} \,|\zeta_4, \vartheta_4, \theta_4\right) + (1 - \pi_4) . G'_4\left(\hat{\beta}_{GI}, \hat{\beta}'_{GP} \,|\zeta'_4, \vartheta'_4, \theta'_4\right)$$

for all $g \in G_4$ **do**

$\omega_g = \sqrt{\left(\frac{\hat{\beta}_{gI}}{max\left|\hat{\beta}_{G_4 I}\right|}\right)^2 + \frac{\left|\hat{\beta}'_{gP}\right|}{max\left|\hat{\beta}'_{G_4 P}\right|}}$ {Assign weights, where $max\left|\hat{\beta}_{G_4 I}\right|$ and $max\left|\hat{\beta}'_{G_4 P}\right|$ are the maximum absolute effects on incidence and prognosis respectively among SNPs assigned to G_4}

end for

$\omega_g = \frac{\omega_g}{\underset{g \in G_4}{max}(\omega_g)}$ {Normalise the weights between zero and 1}

$\mathbb{C} = \emptyset$ {Initialise the set of candidate slopes}

for all $\lambda_j \in \Lambda$ **do**

$\ell_j = \mathbb{S}_{\lambda_j}(G_4, \omega_{G_4})$ {Subsample of size λ_j randomly selected from the set G_4 using the vector of weights ω_{G_4} whose elements are ω_g}

$G^* = \ell_j \cup \{g \in G'_4 \,|P_{gI} < \eta\}$ {The Set of SNPs retained in the next stage of the analysis}

Fit the model-based clusters:

$$f_j\left(\hat{\beta}_{G^*I}, \hat{\beta}'_{G^*P} \,|\pi_1\right) = \pi_1 . G_1\left(\hat{\beta}_{G^*I}, \hat{\beta}'_{G^*P} \,|\zeta_1, \vartheta_1, \theta_1\right) + (1 - \pi_1) . G_2\left(\hat{\beta}_{G^*I}, \hat{\beta}'_{G^*P} \,|\zeta_2, \vartheta_2, \theta_2\right)$$

if $I\left(d_{\mu_1,\mu_2} \leq \delta \,.\, min\left(s_{1I}, s_{1P}, s_{2I}, s_{2P}\right)\right) = 1$ **then**

Fit the linear regression model:

$$M_j : \hat{\beta}'_{G_1 P} = b_j \,.\, \hat{\beta}_{G_1 I} + \epsilon$$

$\mathbb{C} = \mathbb{C} + \hat{b}_j$ {Update the set of slopes by adding the estimated candidate slope}

end if

end for

$\hat{b}_1 = \underset{\hat{b}_j \in \mathbb{C}}{argmin}\left(s\left(\hat{b}_j\right)\right)$ {Identify the optimal slope as the candidate with the minimum standard error}

for all $g \in G$ **do**

$\hat{\beta}_{gP} = \hat{\beta}'_{gP} - \hat{b}_1 \hat{\beta}_{gP}$ {Adjust SNP-prognosis association for all SNPs}

$s_{gP} = \sqrt{\left(s'_{gP}\right)^2 + \hat{b}_1^2 . s_{gI}^2 + \hat{\beta}_{gI}^2 . s\left(\hat{b}_1\right)^2 + s_{gI}^2 . s\left(\hat{b}_1\right)^2}$ {Standard error of the adjusted associations}

end for

return $\hat{\beta}_{GP}$ and $\hat{\beta}_{GP}$

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Underlying assumptions

Our analytic approach assumes the analysed SNPs are independent, do not interact with the confounders, and have linear effects with the incidence and prognosis. Although our framework assumed a linear effect of I on P, equation 2, the size of that effect is not important for our theoretical developments and it might be zero.

The cluster-based model technique theoretically identifies a component distribution that is concentrated around a line (within two-dimensional settings as in our context), which is the first principal component of the corresponding cluster [12]. By definition, the SNP-prognosis associations for genetic variants of the class G_1 are a function of their effects on incidence, see equation 8. Our procedure assumes that the variance of incidence explained by the class G_1 is at least as much as that explained by the class G_2. If this variance condition is true, then the data points of the true class G_1 are more concentrated around the true slope b_1 than the class G_2. In this case, the pattern of the G_1 class can be unambiguously identified by the cluster-based model, and the Slope-Hunter is theoretically valid. This assumption is a less restrictive case of the zero modal pleiotropy assumption (ZEMPA) [17] used in the MR context in which the number of valid instruments are assumed to be larger than any other group sizes of invalid instruments with unique estimates of the causal effect.

Since any correlation between incidence and prognosis should be included in U by definition [4], then our method is robust against the overlap between samples in prognosis GWAS and incidence GWAS. Thus, it is appropriate for one-sample analysis of both incidence and prognosis.

Simulations

We simulated five scenarios, each with $10,000$ independent SNPs under Hardy-Weinberg equilibrium with minor allele frequencies drawn from a uniform distribution over the interval $[0.01, 0.49]$. For all scenarios, both incidence and prognosis were simulated as quantitative traits. The heritability under models shown in equations 1 and 2 was 50%, and the non-genetic confounder, U, explained 40% of variation in both incidence and prognosis, with positive coefficients, β_{UI} and β_{UP}. SNP effects, confounders and residual variation, ε_I and ε_P, were drawn from normal distributions. Data were simulated for $20,000$ unrelated individuals.

In the first scenario (Sc.1), 1% of SNPs (100 SNPs) had effects on incidence only (G_1) explaining 45% of its variation, 9% had effects on both incidence and prognosis (G_2) explaining 5% of variation in incidence, and 1% had effects on prognosis only (G_3). This set up of simulation implies that an index event bias is developed from confounders, with genetic and non-genetic components, that together explain $0.05 + 0.40 = 45\%$ of variation in prognosis. In the second scenario (Sc.2), 5% of SNPs represented G_1, 5% represented G_2, each explaining 25% of variation in incidence, and 5% of SNPs represented G_3. This simulation implies that an index event bias is developed from confounders that explain $0.25 + 0.40 = 65\%$ of variation in prognosis. This simulation reflects a similar scenario to the one discussed by Dudbridge et al [4]. In the third scenario (Sc.3), 1% of SNPs represented G_1, and 9% represented G_2, each explaining 25% of variation in incidence, whereas 1% of SNPs represented G_3. This implies that index event bias is developed

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from confounders that explain 65% of variation in prognosis. The fourth scenario (Sc.4) had 3% of SNPs representing G_1, 7% representing G_2, explaining 15% and 35% of variation in incidence respectively, and 3% representing G_3. For this scenario, an index event bias is developed that explains 75% of variation in prognosis. In the fifth scenario (Sc.5), 1% of SNPs represented G_1, 9% represented G_2, explaining 5% and 45% of variation in incidence respectively, and 1% represented G_3. The set up of this scenario developed an index event bias that explains 85% of variation in prognosis. Table 1 summarises the simulated scenarios. The first scenarios (Sc.1-Sc.3) show how Slope-Hunter performs, and compares its performance to the unadjusted and alternative methods [4], when the assumptions of Slope-Hunter are satisfied. Scenarios Sc.4 and Sc.5 explore performance when these underlying assumptions are not satisfied (i.e. more of the variation in incidence is explained by SNPs in class G_2 than in G_1).

SNP effects on incidence were simulated independently from effects on prognosis for those SNPs that affected both traits (G_2). The simulations of these scenarios were repeated with correlation between SNP effects on incidence and prognosis, whereby effects were drawn from bivariate normal distribution with a particular correlation coefficient of $-0.9, -0.5, 0.5$ and 0.9 for the set of SNPs with effects on both traits. These led to genome-wide genetic correlations between incidence and prognosis of $-0.81, -0.45, 0.45$ and 0.81 for scenarios Sc.1, Sc.3 and Sc.5; $-0.45, -0.25, 0.25$ and 0.45 for scenario Sc.2; and $-0.63, -0.35, 0.35$ and 0.63 for scenario Sc.4 respectively.

Estimated SNP effects on incidence, $\hat{\beta}_{GI}$, were obtained from linear regression of incidence on genotype, and the conditional estimates of SNP effects on prognosis, $\hat{\beta}'_{GP}$, from linear regression of prognosis on genotype and incidence.

When SNP effects on incidence and prognosis are uncorrelated, the index event bias should be exactly the same for various set sizes of G_1 compared with G_2 as the confounding effect would be entirely due to the non-genetic component that is equally simulated across all scenarios. When there is a genetic correlation, at which the genetic component contributes to the confounding effect, the magnitude of the bias should change in a direction determined by the partial confounding effect attributed to the genetic component. For instance in our simulation set-up, when the correlation is positive, the genetic component induces bias that is in the same direction as the bias induced by the non-genetic component, both negative, resulting in a total bias of a greater magnitude than the one induced under no genetic correlation. Under negative correlations, the genetic component induces bias in the opposite direction to the non-genetic component resulting in lower total magnitudes of bias. Under the same level of genetic correlation the contribution of the genetic component to the confounding effect increases as the effect of SNPs causing both incidence and prognosis increases, e.g. from explaining 5%, as in Sc.1, to explaining 25%, as in Sc.2, of variation in incidence, see values of simulated bias at different scenarios shown in Table 2.

If a group of SNPs in the G_2 class affect incidence and prognosis only through a common exposure, E, as depicted in Figure 3, then their genetic effects on incidence should perfectly correlate with their effects on prognosis, leading to constant proportional relationship for their SNP-prognosis to SNP-incidence associations. In this case, if the class G_2 explains more of the variation in incidence than the class G_1, the Slope-Hunter may be severely biased because it would completely swap the classes rather than having affordable misclassification error as expected when its assumptions are

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slightly violated. We simulated a sixth scenario (Sc.6) introducing this case, whereby 5% of SNPs represented the class G_1, 10% represented G_2, explaining 25% and 50% of variation in incidence respectively, and 5% representing G_3. The class G_2 was dominated by a subset of SNPs (G_2^*: 70% of G_2 that explained 35% of the total variation of incidence) whose effects on incidence and prognosis traits occur only via a common exposure. For a SNP g_2^* from the subset G_2^*, the ratio of SNP-prognosis to SNP-incidence associations can be expressed as follows:

$$\frac{\beta_{g_2^* P}}{\beta_{g_2^* I}} = \frac{b_1\,\beta_{EI} + \beta_{EP}}{\beta_{EI}} = b_1 + \frac{\beta_{EP}}{\beta_{EI}}, \tag{9}$$

where b_1 is the true confounding effect between incidence and prognosis, whereas β_{EI} and β_{EP} are the effects of exposure on incidence and prognosis respectively. This suggests a fixed ratio of SNP-prognosis to SNP-incidence associations for the group G_2^*, assuming small individual genetic effects as typically applies for polygenic traits. The remaining SNPs in the G_2 class were simulated to have uncorrelated genetic effects on incidence and prognosis.

We performed 1000 simulations for each scenario and reported mean of differences between estimated adjustment factors and the true index event bias. The type-1 error rates, at $p < 0.05$, of SNP effects on prognosis were evaluated. Since the index event bias is proportional to the effect on incidence, as shown in equation 5, type-1 error rates vary among SNPs with different effects on incidence. Therefore, we estimated: the mean type-1 error over all SNPs with no effect on prognosis (i.e. true classes of G_1 and G_4); the mean type-1 error over SNPs with effects on incidence only (i.e. true class of G_1) because G_4 has no index event bias and its SNPs can dominate G_1, when combined, due to class sizes. We estimated the family-wise type-1 error over the true class of G_1, as the proportion of simulations in which at least one variant had $p < 0.05$ after Bonferroni multiple-testing adjustment for the number of SNPs. The mean power over all SNPs with effects on prognosis (true classes of G_2 and G_3), and over SNPs with effects on both incidence and prognosis (true classes of G_2) were estimated. The mean absolute bias and mean square error across all SNPs, and across SNPs with effects on incidence (true classes of G_1 and G_2) were estimated.

Results of the Slope-Hunter (SH estimator) method were compared with the unadjusted estimator $\hat{\beta}'_{GP}$, estimator of the method of Dudbridge et al (2019) with Hedges-Olkin adjustment (H-O estimator) and with simulation extrapolation adjustment (SIMEX estimator) for regression dilution [4]. Because H-O and SIMEX results were almost identical, we only presented H-O results. Furthermore, the individual SNP with highest type-1 error for the unadjusted estimator is identified and compared with the type-1 error of estimators of the adjustment methods. We identified SNPs with the greatest increase and decrease in power between the unadjusted estimator and all estimators of the compared adjustment methods, SH and H-O. The mean of maximum absolute bias is also compared between the unadjusted and adjusted estimators.

To evaluate Slope-Hunter's estimations of the G_1 class membership probabilities, we calculated the mean probability for the SNPs identified as the G_1 class. Moreover, the misclassification error rate [18, 19] was obtained by comparing Slope-Hunter's assignments of the SNPs to the G_1 class with their true class status.

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3 Results

Simulation results

Table 2 shows means of differences between adjustment factors estimated using each of the compared methods, H-O ([4]) and SH (our approach), and the true index event bias across 1000 simulations at different scenarios Sc.1 - Sc.5 at various levels of genetic correlations. Our method, the SH, gave unbiased estimates of the adjustment factor when the proportion of variation in incidence explained by the class G_1 was larger than (Sc.1) and equal (Sc.2) to those explained by the class G_2. The corresponding H-O estimates were biased, with bias increasing as the genetic correlations became stronger. For Sc.1 and Sc.2, the type-1 error rates obtained from the unadjusted estimator as well as H-O and SH estimators were close to the nominal level, 0.05, when averaged over all SNPs, with slightly lower error rates for SH, see Tables 3 and 4. Since the majority of SNPs did not affect incidence, they did not suffer from index event bias. Among those with effects on incidence, for which there was bias, the type-1 error was inflated for the unadjusted analysis, ranging from 0.60 to 0.69 in Sc.1 and from 0.09 to 0.49 in Sc.2. For the H-O estimator, the type-1 error of SNPs affecting incidence was also inflated, ranging 0.08 to 0.14 in Sc.1 and from 0.15 to 0.35 for Sc.2 under genetic correlations. The type-1 error rate for our approach was consistently close to the correct rate, $0.05 - 0.06$, even in the presence of genetic correlation between incidence and prognosis in both scenarios Sc.1 and Sc.2. The type-1 error for the individual SNP with the highest error under the unadjusted analysis was high, 1 in Sc.1 and $0.43 - 1$ in Sc.2, but was substantially reduced using our procedure under all levels of genetic correlations, achieving the correct rate, 0.05, in Sc.1, and ranging from 0.06 to 0.14 in Sc.2. The H-O failed to reduce the error rate except under no genetic correlation. There was a similar pattern for the family-wise error rate. Overall, there were small to moderate drops in power for all adjustment methods compared to the unadjusted analysis, except under strong positive correlation where there was an increase. Our method consistently achieved higher power rates than the H-O method under all levels of genetic correlations. For some individual SNPs the power rate was low under the unadjusted analysis but was substantially increased under all adjustment approaches, with the greatest increase under the SH method when there was positive correlation between incidence and prognosis. Under negative correlation, the H-O method had greater increase in power for some individual SNPs compared to SH method, and similar increase when there was no genetic correlation. Our procedure had the lowest absolute bias compared to the unadjusted as well as the other adjusted analysis under all levels of genetic correlations. There were similar patterns for the mean square error.

All methods consistently performed less well as the proportion of variation in incidence explained by the G_1 class decreased in the presence of genetic correlation. However, the SH estimator consistently outperformed the other estimators providing the closest estimates to the true bias and the lowest type-1 error rates, see Table 2 and Tables 5-7. Under no genetic correlation, the SH method performed as well as the H-O method when G_1 explains at least as much variation in incidence as G_2 as in Sc.1 - Sc.3, see Tables 2 - 5. In Sc.3, the SH method consistently outperformed the other methods - in terms of bias and type-1 error - under all levels of genetic correlation. SH performed less well under strong genetic correlation in Sc.3 compared to Sc.1 (i.e. performed less well where the variance of incidence explained

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by the G_1 class was lower). SH performed less well under strong genetic correlation in Sc.3 compared to Sc.2 (i.e. performed less well where the number of SNPs in the G_1 class was lower). Tables 6 and 7 show results of type-1 error, power, absolute bias and mean square error for Sc.4 and Sc.5 respectively. As expected, the SH estimator was more biased as the class G_1 explained less variation in I than the class G_2, with worse performance under stronger genetic correlation. But, the bias in the SH estimator was lower than in H-O estimator for small and moderate index event bias (ranging from -0.5 to 0). Our approach provided lower type-1 error rates than the unadjusted analyses as well as the H-O method except under strong correlation in Sc.4. The SH estimator provided equivalent power, compared with the alternatives, except under strong correlation, where it had slightly lower power in Sc. 4 and Sc.5. The absolute bias and mean square errors showed similar pattern to the results of type-1 error. In Sc.6, the SH estimator was severely biased providing worse type-1 error rates, 0.56 versus 0.36, with lower power, 0.25 versus 0.45, compared with the unadjusted estimator, see Table S1 in the supplementary material.

The mean probabilities of memberships assignments to the cluster G_1 obtained by the SH were similar, ranging between 0.80 to 0.90, in Sc.1 and Sc.3. The classification error rates were slightly lower in Sc.3 compared with Sc.1, reflecting better identification of variants belonging to the true G_1 class, see Table S2 in the supplementary material. Figures 8 and 9 present uncertainty plots for the variants assigned to the G_1 cluster by the SH method in four simulations selected randomly from the 1000 simulations of Sc.1 with -0.9 and 0.9 correlations respectively. The misclassified SNPs, indicated by vertical black lines, were the ones with the highest uncertain identification. Figure S1 in the supplementary material shows uncertainty plots for the Sc.6, in which almost all variants of the G_1 cluster were misclassified regardless of their uncertainty levels.

4 Discussion

Analysis of causal effects on prognosis, such as subsequent disease events, severity and survival time, is increasingly motivated by many large collections of GWAS for disease cases. Such case-only studies are liable to index event bias, whereby independent causes of the incidence become correlated when selecting only on cases and then may confound analysis of prognosis. We have proposed an approach that overcomes a major disadvantage of previous methods, and showed that it provides unbiased estimates of SNP-prognosis associations in a variety of situations including the presence of genetic correlations between incidence and prognosis. Our approach aims to identify the set of SNPs with effects only on incidence and uses it to estimate and adjust for the index event bias induced by the confounder effects. Therefore, our approach is robust against the violation of the 'InCLUDE' assumption, that is the direct genetic effects on prognosis are assumed to be linearly uncorrelated with effects on incidence. Our analytic approach assumes the analysed SNPs are independent, do not interact with the confounders, and have linear effects with the incidence and prognosis. Moreover, it assumes that the variance of incidence explained by the class G_1 is at least as much as that explained by the class G_2. Under satisfaction of these underlying assumptions, our procedure is highly adaptive in dealing with genetic correlations, and can maintain excellent trade-off between type-1 error rates and power and produce lower mean square error compared to the other methods. Independence of the analysed SNPs can be achieved

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by selecting from GWAS through LD-pruning prioritising by p-values of SNP-incidence associations. However, if traits are generated under non-linear models or the variance of incidence explained by the SNPs affecting only incidence is extremely small, a biased adjustment factor might be derived by our procedure leading to inexact correction for the index event bias.

We simulated an index event bias by analysing prognosis conditional on a continuous incidence trait. Dudbridge et. al. (2018) showed that similar index event bias is induced when incidence trait is a binary disease and prognosis is analysed in cases only [4]. Our simulations showed that our approach consistently achieved the minimum type-1 error rates, with slightly less power on average and considerably higher power for some individual SNPs, compared with the unadjusted analysis. Compared with alternative methods, our procedure had lower type-1 error rates and higher average power under various levels of genetic correlations between incidence and prognosis. All methods had worse type-1 error rates under genetic correlations as the proportion of incidence variance explained by SNPs affecting incidence only reduced. However our approach had better type-1 error rates than the alternatives when this proportion was not very small, except under strong correlations where it produced worse type-1 error than the unadjusted analysis but better type-1 error and less biased estimates than the compared methods.

Our method relies on obtaining an estimate of the bias adjustment factor using information from the SNPs that only affect incidence, the G_1 class. Since identification of this class is more accurate when its variants are more concentrated around the cluster's slope [12], then our procedure produced biased estimates as the SNPs in this group explained less of variation in incidence than the variants affecting both incidence and prognosis. The unadjusted analysis similarly performed worse as the proportion of incidence's variance explained by SNPs affecting only incidence was decreased, while the corresponding proportion for the SNPs affecting both incidence and prognosis was increased. This could be due to the increase in confounder effects, and hence the bias, resulting from increased effects of SNPs influencing both incidence and prognosis, the G_2 class. Increasing effects of SNPs in the G_2 class on incidence in the presence of genetic correlation between incidence and prognosis caused more violation of the 'InCLUDE' assumption, a critical assumption for the H-O estimator [4], leading to more biased estimates of these methods. The SH estimator may provide less good performance in index event bias correction when the misclassification in the G_1 cluster severely influences its pattern. This may occur if the variants of G_2, that are misclassified to the G_1 cluster, explain more variation in incidence than the class G_1 (as in Sc.4 and Sc.5); or explain equal variation in I but the size of the G_1 cluster is small, as in Sc.3, such that a misclassified SNP would have a bigger effect on its pattern. In either case, the influence of misclassification is more pronounced under stronger genetic correlation, which applies only, by definition, to SNPs affecting both incidence and prognosis.

Our procedure requires user choices for the input parameters, Λ, η and δ. These choices impact identification of the class of SNPs with effects only on incidence, G_1. For example, retaining a sub-sample of only 1% of SNPs with no effects on neither incidence nor prognosis, the G_4 class, may not be sufficient to aid pattern recognition of the G_1 cluster in the dimensional space. Therefore, it may be beneficial to retain larger sub-sample of the class G_4, but too large sub-samples may distort pattern of the class G_1 leading to poor cluster identification. Fitting cluster-based models

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iteratively using a number of proportions given by the set Λ allows users to specify many potential sub-sample sizes to be retained in the analysis and the algorithm can then pick the best size as the one leading to the highest precision of estimate of the adjustment factor. Inclusion of more SNPs is the final stage of analysis as potential members of either the class G_1 or G_2, when more lenient p-value threshold, η, is used, could lead to improving efficiency due to potential increase in underlying SNPs of G_1 class. But, it might also result in including a fraction of SNPs with no effects on incidence in the G_1 class, whose pattern may be obscured if this fraction is large. Under the assumptions of our procedure, the SNPs in G_2 class are expected to be scattered around the slope, b_1, with higher variations than the SNPs in G_1 class. Then, a valid cluster solution should have a relatively small Euclidean distance between the means of clusters G_1 and G_2. Our simulations showed that this distance could be maximally equal to the minimum of standard deviations of both clusters on each dimension. However, the parameter δ is used as a multiplier of the minimum standard deviation that can be set by the user to define valid cluster solutions for different data structures.

The main idea of our procedure can be exploited in future in the context of the MR analysis using a large number of genetic variants including invalid instruments, particularly for experiments in which effects of instruments on exposure and outcome are correlated[20]. This potential direction may be beneficial in robustly estimating causal effects, checking violation of MR assumptions, and providing probabilistic identification of the valid instruments in a given problem. A few methods have been recently developed with a conceptual similarity to the Slope-Hunter in the context of MR analysis, that is they aim to identify the valid instruments, and then use this class of genetic variants to estimate causal effect of an exposure on outcome. These include the MR-mix [17] and CAUSE [21] methods. The Slope-Hunter approach can be adapted in future to be used in conjunction with these methods to form a consensus results of GWAS hits for a trait since MR assumptions can not be exactly verified. For instance, the MR-mix method relies on the ZEMPA assumption implying that the number of valid instruments are assumed to be larger than any other group sizes of invalid instruments with unique estimates of the causal effect. The Slope-Hunter has a less restrictive assumption implying that the group of SNPs affecting incidence only explains at least as much variation in incidence as the group of SNPs affecting both incidence and prognosis.

Our study has several strengths. It provides a novel framework that uses a cluster-based model approach to correct for index event bias even in the presence of genetic correlations between incidence and prognosis. Our approach provides estimates for the membership probability of each SNP to each cluster allowing us to evaluate the obtained cluster solutions. We evaluated our developed approach in a variety of situations including different levels of genetic correlations, leading to different magnitudes of genetic confounders, and different number of SNPs with effects only on incidence. Our study compared the performance of the SH method with the unadjusted analysis as well as with other alternative methods in terms of many statistical criteria including type-1 error, power, bias and mean squared error. On the other hand, there are a number of limitations. Although we have examined the SH performance in a wide range of situations, including violating the 'InCLUDE' assumption and larger effects for the G_2 class, we have not examined the sensitivity to non-linearity or to interaction between confounder and variant's effects. We have not examined sensitivity to the user-set parameters, and these may be refined in light of how the method performs in practice. There is not a

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single criterion for validity of the slope-hunter approach, as it will depend on how separated the classes G_1 and G_2 are and whether the class G_1 is correctly identified. However, the performance of the SH is influenced by the identified pattern of the G_1 class, and hence its slope, rather than the exact accuracy of classifying the G_1 variants. Therefore, the SH can afford a reasonable amount of misclassification error as long as the misclassified SNPs do not severely distort the estimated pattern of the target class. Assumptions of the Slope-Hunter are not testable, but the estimated probabilities of fitted cluster solutions for G_1 SNPs can be used for diagnosis purposes to assess the method performance.

We have proposed an approach for adjusting for index event bias in GWAS of subsequent events even in the presence of genetic correlation between incidence and prognosis. We recommend that this approach is used in GWAS of events after incidence of a disease, to minimise the bias due to conditioning on incidence. This approach is also recommended for subsequent use of GWAS results, such as in MR analyses of the effect of exposure on prognosis. All procedures described in this manuscript have been implemented into an open source R package named 'Slope-Hunter'.

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5 Tables

Table 1: Descriptions of simulated scenarios by means of class sizes for SNPs with effects on incidence only (G_1) and SNPs with effects on both incidence and prognosis (G_2), and their corresponding explained variation in incidence

Scenario	G_1		G_2		% P's variance explained by confounders
	Size	% I's variance explained	Size	% I's variance explained	
Sc.1	1%	45%	9%	5%	45%
Sc.2	5%	25%	5%	25%	65%
Sc.3	1%	25%	9%	25%	65%
Sc.4	3%	15%	7%	35%	75%
Sc.5	1%	5%	9%	45%	85%

Abbreviations: I = incidence; P = prognosis; G_1 = true class of SNPs with effects on incidence only; G_2 = true class of SNPs with effects on incidence and prognosis.

In all scenarios, $10,000$ independent SNPs were simulated under Hardy-Weinberg equilibrium with minor allele frequencies drawn from a uniform distribution over the interval $[0.01, 0.49]$. Both incidence and prognosis were simulated as quantitative traits. The heritability was 50%, and the non-genetic confounder, U, explained 40% of variation in both incidence and prognosis. SNP effects, confounders and residual variation, ε_I and ε_P, were drawn from normal distributions. Data were simulated for $20,000$ unrelated individuals. In each scenario, an index even bias is developed form genetic and non-genetic confounders that both explain certain proportion - shown in the last column - of variation in prognosis

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Table 2: Means and standard deviations (SD) of the differences between estimated adjustment factors using H-O and Slope-Hunter methods, and the true index event bias (B) over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitative trait, for five scenarios (Sc.1 - Sc.5) described in the first and second rows

		% G_1 SNPs (proportion of variation in incidence explained by G_1) Vs. % G_2 SNPs (proportion of variation in incidence explained by G_2)									
		Sc.1: 1% (0.45) Vs. 9% (0.05)		**Sc.2**: 5% (0.25) Vs. 5% (0.25)		**Sc.3**: 1% (0.25) Vs. 9% (0.25)		**Sc.4**: 3% (0.15) Vs. 7% (0.35)		**Sc.5**: 1% (0.05) Vs. 9% (0.45)	
G. cor	Method	Mean diff. (SD)	Mean B (SD)	Mean diff. (SD)	Mean B (SD)	Mean diff. (SD)	Mean B (SD)	Mean diff. (SD)	Mean B (SD)	Mean diff. (SD)	Mean B (SD)
-0.90	H-O	-0.08 (0.01)	-0.33 (0.01)	-0.43 (0.02)	-0.17 (0.01)	-0.43 (0.02)	-0.17 (0.01)	-0.62 (0.02)	-0.08 (0.02)	-0.81 (0.03)	0.01 (0.03)
	SH	-0.01 (0.02)		0.01 (0.06)		-0.12 (0.30)		-0.45 (0.51)		-0.89 (0.07)	
-0.50	H-O	-0.04 (0.01)	-0.35 (0.01)	-0.24 (0.02)	-0.26 (0.01)	-0.23 (0.02)	-0.26 (0.01)	-0.33 (0.02)	-0.22 (0.02)	-0.43 (0.03)	-0.17 (0.03)
	SH	-0.01 (0.01)		0.02 (0.08)		0.05 (0.12)		0.02 (0.07)		0.14 (0.22)	
Zero	H-O	0 (0.01)	-0.37 (0.01)	0 (0.02)	-0.37 (0.01)	0 (0.02)	-0.37 (0.01)	0 (0.02)	-0.37 (0.02)	0 (0.03)	-0.37 (0.03)
	SH	0 (0.002)		0.02 (0.02)		0 (0.01)		0.04 (0.11)		0.37 (0.12)	
0.50	H-O	0.05 (0.01)	-0.39 (0.01)	0.23 (0.02)	-0.47 (0.01)	0.23 (0.02)	-0.48 (0.01)	0.31 (0.02)	-0.51 (0.02)	0.40 (0.03)	-0.55 (0.03)
	SH	0 (0.003)		0.02 (0.07)		0 (0.04)		0.13 (0.16)		0.45 (0.17)	
0.90	H-O	0.08 (0.01)	-0.41 (0.01)	0.40 (0.02)	-0.55 (0.01)	0.40 (0.02)	-0.56 (0.01)	0.55 (0.02)	-0.62 (0.02)	0.70 (0.03)	-0.69 (0.03)
	SH	0 (0.004)		-0.03 (0.02)		0.28 (0.40)		0.72 (0.26)		0.81 (0.05)	

Abbreviations: G_1 = true class of SNPs with effects on incidence only; G_2 = true class of SNPs with effects on incidence and prognosis; G. cor = genetic correlation of SNP effects on incidence and prognosis; H-O = estimator of the method of Dudbridge et al (2019) with Hedges-Olkin adjustment for regression dilution; SH = 'Slope-Hunter' estimator; Mean diff. = mean of differences between estimated adjustment factor using corresponding estimator and the true index event bias; SD = standard deviation; B = true index event bias.

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Table 3: Type-1 error and power at $p < 0.05$, absolute bias and mean square error over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitiative trait, for Sc.1: 1% of SNPs have effects on incidence only (explaining 45% of its variation), 1% on prognosis only and 9% on both incidence and prognosis (explaining 5% of variation in incidence). Heritability of incidence and prognosis is 50% with the genetic correlation between SNP effects on incidence and prognosis shown in the first column. Non-genetic common factors explain 40% of variation in both incidence and prognosis. The index event bias explains $\sim 45\%$ of variation in prognosis

G. cor	Method	Type-1 error (%)		Power (%)		Absolute bias ($\times 10^{-3}$)		MSE ($\times 10^{-5}$)		MAB	FWE	HE	IP	DP
		All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2					
	Unadjusted	5.6	60.5	34.3	28.5	4.9	6.3	4.1	9.0	76.6	100.0	100.0	12.1	92.5
-0.90	H-O	5.1	13.1	22.4	15.6	5.3	5.5	4.5	4.9	27.6	8.2	28.0	15.4	36.2
	SH	5.0	5.2	24.6	17.9	5.1	5.2	4.2	4.2	26.2	4.1	5.2	14.8	50.6
	Unadjusted	5.7	62.7	32.4	26.4	4.9	6.5	4.1	9.6	80.6	100.0	100.0	29.4	72.6
-0.50	H-O	5.1	7.6	24.0	17.2	5.2	5.3	4.3	4.4	26.6	4.2	12.2	47.0	13.6
	SH	5.0	5.0	24.8	18.1	5.1	5.1	4.1	4.1	26.1	4.4	4.8	45.7	17.3
	Unadjusted	5.7	65.2	28.9	22.4	4.8	6.6	4.1	10.4	85.5	100.0	100.0	19.9	73.9
Zero	H-O	5.0	5.2	24.8	18.1	5.1	5.1	4.1	4.1	26.1	4.2	5.1	49.4	24.5
	SH	5.0	4.9	24.7	18.1	5.1	5.1	4.1	4.1	26.1	4.2	5.0	49.5	24.4
	Unadjusted	5.7	67.4	24.5	17.6	4.8	6.8	4.1	11.2	90.4	100.0	100.0	9.6	72.5
0.50	H-O	5.1	8.2	24.8	18.1	5.0	5.0	3.9	4.0	25.8	6.4	9.6	44.4	34.3
	SH	5.0	4.9	24.8	18.1	5.1	5.1	4.1	4.0	26.1	4.8	4.8	49.2	30.6
	Unadjusted	5.7	69.0	19.4	11.9	4.7	7.0	4.1	12.0	94.3	100.0	100.0	26.7	86.0
0.90	H-O	5.1	14.4	24.0	17.1	4.9	5.1	3.8	4.1	27.0	25.5	15.6	69.7	77.7
	SH	5.0	5.0	24.8	18.2	5.1	5.1	4.0	4.0	26.1	4.7	5.7	76.7	73.2

Abbreviations: G. cor = Genetic correlation of SNP effects on incidence and prognosis; MSE = Mean square error; G_1 & G_2 = All SNPs affecting incidence; MAB = mean of the maximum absolute bias over simulations ($\times 10^{-3}$); FWE = family-wise type-1 error (%); HE = Type-1 error of the SNP with highest error for the unadjusted estimator (%); IP = Power of the SNP with greatest increase in power between the unadjusted and adjusted estimators; DP = Power of the SNP with greatest decrease in power between the unadjusted and adjusted estimators; H-O = the 'Hedges-Olkin' estimator; SH = 'Slope-Hunter' estimator.

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Table 4: Type-1 error and power at $p < 0.05$, absolute bias and mean square error over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitative trait, for Sc.2: 5% of SNPs have effects on incidence only (explaining 25% of its variation), 5% on prognosis only and 5% on both incidence and prognosis (explaining 25% of variation in incidence). Heritability of incidence and prognosis is 50% with the genetic correlation between SNP effects on incidence and prognosis shown in the first column. Non-genetic common factors explain 40% of variation in both incidence and prognosis. The index event bias explains $\sim 65\%$ of variation in prognosis

G. cor	Method	Type-1 error (%)		Power (%)		Absolute bias ($\times 10^{-3}$)		MSE ($\times 10^{-5}$)		MAB	FWE	HE	IP	DP
		All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2					
	Unadjusted	5.2	8.9	61.0	62.6	5.3	5.9	4.4	5.4	27.0	8.8	43.4	12.7	100.0
-0.90	H-O	5.9	21.8	42.8	32.9	6.6	10.2	7.1	16.2	43.6	89.8	97.0	63.5	5.7
	SH	5.0	5.3	58.2	57.5	5.3	5.4	4.4	4.6	26.9	5.9	7.3	26.4	97.4
	Unadjusted	5.6	15.1	62.2	64.1	5.2	6.7	4.2	7.0	29.3	37.7	80.8	6.9	99.6
-0.50	H-O	5.4	11.5	52.6	49.8	5.9	7.3	5.5	8.3	31.9	13.6	60.1	100.0	8.8
	SH	5.1	5.9	58.6	58.3	5.2	5.4	4.3	4.6	26.5	5.6	14.5	83.3	73.9
	Unadjusted	6.2	25.8	61.5	60.8	5.0	8.1	4.1	10.3	37.6	99.5	99.4	5.1	95.8
Zero	H-O	5.0	5.0	57.6	56.3	5.1	5.2	4.1	4.2	25.7	5.0	4.6	97.3	7.9
	SH	5.0	5.0	57.8	56.5	5.1	5.1	4.0	4.2	25.5	5.1	5.7	95.8	7.2
	Unadjusted	6.9	38.2	60.7	56.5	4.8	9.9	4.0	15.3	41.6	100.0	100.0	4.4	98.3
0.50	H-O	5.5	14.7	59.1	55.2	4.6	6.4	3.4	6.4	27.8	34.1	77.3	75.3	43.9
	SH	5.1	6.0	57.0	54.7	5.0	5.2	3.9	4.4	25.4	7.5	9.9	97.4	8.5
	Unadjusted	7.4	48.6	53.8	40.0	4.5	11.4	4.1	20.7	49.3	100.0	100.0	5.9	77.9
0.90	H-O	6.7	34.9	56.6	46.3	4.4	8.9	3.4	12.6	39.4	100.0	100.0	31.5	56.0
	SH	5.0	5.1	57.0	55.8	5.0	5.1	3.9	4.1	25.3	5.0	5.6	98.9	6.1

Abbreviations: G. cor = Genetic correlation of SNP effects on incidence and prognosis; MSE = Mean square error; G_1 & G_2 = All SNPs affecting incidence; MAB = mean of the maximum absolute bias over simulations ($\times 10^{-3}$); FWE = family-wise type-1 error (%); HE = Type-1 error of the SNP with highest error for the unadjusted estimator (%); IP = Power of the SNP with greatest increase in power between the unadjusted and adjusted estimators; DP = Power of the SNP with greatest decrease in power between the unadjusted and adjusted estimators; H-O = the 'Hedges-Olkin' estimator; SH = 'Slope-Hunter' estimator.

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Table 5: Type-1 error and power at $p < 0.05$, absolute bias and mean square error over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitiative trait, for Sc.3: 1% of SNPs have effects on incidence only (explaining 25% of its variation), 1% on prognosis only and 9% on both incidence and prognosis (explaining 25% of variation in incidence). Heritability of incidence and prognosis is 50% with the genetic correlation between SNP effects on incidence and prognosis shown in the first column. Non-genetic common factors explain 40% of variation in both incidence and prognosis. The index event bias explains $\sim 65\%$ of variation in prognosis

G. cor	Method	Type-1 error (%)		Power (%)		Absolute bias ($\times 10^{-3}$)		MSE ($\times 10^{-5}$)		MAB	FWE	HE	IP	DP
		All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2					
	Unadjusted	5.2	22.3	54.4	51.7	5.3	5.8	4.4	5.4	31.9	61.5	99.7	8.9	95.4
-0.90	H-O	5.5	49.5	28.3	23.1	6.6	9.7	7.1	16.2	72.0	100.0	100.0	37.8	6.2
	SH	5.1	14.2	44.3	40.7	5.8	6.9	5.7	10.5	43.2	20.2	19.8	20.8	72.2
	Unadjusted	5.4	38.3	55.6	53.0	5.1	6.4	4.2	7.0	45.0	100.0	100.0	12.3	99.8
-0.50	H-O	5.3	29.5	42.2	38.4	5.9	7.1	5.5	8.3	40.8	95.0	100.0	81.1	7.0
	SH	5.1	10.4	47.8	44.5	5.4	5.7	4.5	5.4	30.4	20.2	22.3	55.6	56.8
	Unadjusted	5.6	54.9	54.3	51.3	4.9	7.7	4.1	10.4	62.5	100.0	100.0	12.2	98.2
Zero	H-O	5.0	5.1	48.5	45.1	5.1	5.2	4.1	4.2	25.8	5.0	4.3	94.1	10.9
	SH	5.0	5.0	48.5	45.1	5.1	5.2	4.1	4.2	25.8	5.1	4.5	94.1	11.6
	Unadjusted	5.7	67.0	49.9	46.3	4.7	9.3	4.0	15.4	79.9	100.0	100.0	8.7	99.8
0.50	H-O	5.4	37.2	49.0	45.5	4.6	6.2	3.4	6.4	39.8	100.0	72.0	83.0	68.1
	SH	5.0	5.5	48.3	44.9	5.0	5.2	3.9	4.2	25.9	4.9	6.2	98.4	7.4
	Unadjusted	5.8	74.0	35.7	30.3	4.5	10.8	4.1	20.8	93.4	100.0	100.0	7.1	90.8
0.90	H-O	5.7	64.0	41.0	36.2	4.3	8.5	3.4	12.6	67.6	100.0	98.9	19.7	77.8
	SH	5.3	30.3	41.7	37.5	5.0	8.5	4.8	17.0	64.2	36.5	36.5	80.1	46.5

Abbreviations: G. cor = Genetic correlation of SNP effects on incidence and prognosis; MSE = Mean square error; G_1 & G_2 = All SNPs affecting incidence; MAB = mean of the maximum absolute bias over simulations ($\times 10^{-3}$); FWE = family-wise type-1 error (%); HE = Type-1 error of the SNP with highest error for the unadjusted estimator (%); IP = Power of the SNP with greatest increase in power between the unadjusted and adjusted estimators; DP = Power of the SNP with greatest decrease in power between the unadjusted and adjusted estimators; H-O = the 'Hedges-Olkin' estimator; SH = 'Slope-Hunter' estimator.

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Table 6: Type-1 error and power at $p < 0.05$, absolute bias and mean square error over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitiative trait, for Sc.4: 3% of SNPs have effects on incidence only (explaining 15% of its variation), 3% on prognosis only and 7% on both incidence and prognosis (explaining 35% of variation in incidence). Heritability of incidence and prognosis is 50% with the genetic correlation between SNP effects on incidence and prognosis shown in the first column. Non-genetic common factors explain 40% of variation in both incidence and prognosis. The index event bias explains $\sim 75\%$ of variation in prognosis

G. cor	Method	Type-1 error (%)		Power (%)		Absolute bias ($\times 10^{-3}$)		MSE ($\times 10^{-5}$)		MAB	FWE	HE	IP	DP
		All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2					
	Unadjusted	5.0	6.0	58.1	58.6	5.4	5.5	4.6	4.8	27.3	5.5	13.4	20.2	99.8
-0.90	H-O	5.8	30.4	31.6	24.2	7.3	13.0	9.0	26.9	56.4	99.9	100.0	71.2	5.0
	SH	5.5	20.8	41.6	38.1	7.2	11.9	9.7	31.1	53.2	47.1	48.0	49.1	68.7
	Unadjusted	5.2	11.8	59.8	60.8	5.2	6.2	4.3	6.1	27.6	10.3	62.0	4.4	100.0
-0.50	H-O	5.4	16.4	48.7	47.2	6.2	8.7	6.2	11.8	37.6	35.5	84.9	90.7	5.0
	SH	5.0	5.6	56.4	56.3	5.2	5.4	4.3	4.6	26.6	5.8	10.8	27.2	85.0
	Unadjusted	5.7	25.8	61.7	62.4	5.0	8.2	4.1	10.4	33.8	96.0	98.9	4.7	99.8
Zero	H-O	5.0	5.0	56.5	56.3	5.1	5.2	4.1	4.3	25.7	5.0	4.8	78.6	10.3
	SH	5.1	6.9	57.0	56.9	5.1	5.5	4.1	4.9	26.4	11.0	15.1	72.1	20.9
	Unadjusted	6.3	42.8	61.9	61.1	4.6	10.6	4.0	18.0	47.6	100.0	100.0	9.5	6.4
0.50	H-O	5.6	24.1	60.4	59.4	4.5	7.6	3.3	9.1	34.3	90.5	97.3	90.5	80.5
	SH	5.2	11.4	58.1	57.7	4.7	6.0	3.6	6.3	28.0	21.7	28.7	87.6	87.4
	Unadjusted	6.7	57.1	51.2	43.7	4.2	12.9	4.2	26.7	58.8	100.0	100.0	17.7	98.0
0.90	H-O	6.6	52.3	52.9	46.3	4.1	11.6	3.7	21.8	53.4	100.0	100.0	4.9	100.0
	SH	6.7	56.9	48.5	41.0	4.8	14.9	5.7	38.0	67.6	88.3	88.4	88.1	19.6

Abbreviations: G. cor = Genetic correlation of SNP effects on incidence and prognosis; MSE = Mean square error; G_1 & G_2 = All SNPs affecting incidence; MAB = mean of the maximum absolute bias over simulations ($\times 10^{-3}$); FWE = family-wise type-1 error (%); HE = Type-1 error of the SNP with highest error for the unadjusted estimator (%); IP = Power of the SNP with greatest increase in power between the unadjusted and adjusted estimators; DP = Power of the SNP with greatest decrease in power between the unadjusted and adjusted estimators; H-O = the 'Hedges-Olkin' estimator; SH = 'Slope-Hunter' estimator.

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Table 7: Type-1 error and power at $p < 0.05$, absolute bias and mean square error over 1000 simulations of 10,000 independent SNPs, conditional on incidence as a quantitiative trait, for Sc.5: 1% of SNPs have effects on incidence only (explaining 5% of its variation), 1% on prognosis only and 9% on both incidence and prognosis (explaining 45% of variation in incidence). Heritability of incidence and prognosis is 50% with the genetic correlation between SNP effects on incidence and prognosis shown in the first column. Non-genetic common factors explain 40% of variation in both incidence and prognosis. The index event bias explains $\sim 85\%$ of variation in prognosis

G. cor	Method	Type-1 error (%)		Power (%)		Absolute bias ($\times 10^{-3}$)		MSE ($\times 10^{-5}$)		MAB	FWE	HE	IP	DP
		All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2	All SNPs	G_1 & G_2					
	Unadjusted	5.0	5.0	56.3	56.3	5.5	5.5	4.8	4.8	27.8	5.2	8.2	5.0	100.0
-0.90	H-O	5.4	37.3	22.0	19.2	8.0	16.0	11.3	40.8	77.7	100.0	5.4	66.8	4.3
	SH	5.4	39.3	20.6	17.9	8.5	17.5	12.7	48.6	85.3	100.0	5.4	71.3	7.3
	Unadjusted	5.1	8.9	60.3	60.6	5.3	5.9	4.4	5.5	27.1	5.7	45.5	8.2	99.9
-0.50	H-O	5.2	21.3	46.6	46.2	6.6	10.1	7.1	16.2	45.6	53.1	99.0	95.4	4.7
	SH	5.1	12.7	59.1	59.4	5.5	6.8	4.8	7.7	31.1	19.6	62.4	28.4	89.6
	Unadjusted	5.2	25.2	61.5	61.7	5.0	8.2	4.1	10.4	34.7	77.9	99.8	11.0	96.7
Zero	H-O	5.0	5.0	56.1	56.0	5.1	5.2	4.1	4.3	25.6	5.4	5.2	95.7	4.8
	SH	5.2	25.5	61.4	61.6	5.0	8.4	4.2	11.2	35.7	73.5	94.2	17.9	90.1
	Unadjusted	5.5	47.7	61.1	60.7	4.5	11.6	4.1	20.9	52.9	100.0	100.0	9.0	85.7
0.50	H-O	5.3	34.0	59.6	59.0	4.4	9.1	3.4	12.9	41.6	99.5	100.0	68.0	19.0
	SH	5.4	39.8	60.1	59.7	4.5	10.2	3.8	16.9	46.9	89.3	92.4	36.1	65.7
	Unadjusted	5.7	67.7	50.2	47.6	3.9	14.6	4.4	33.8	75.4	100.0	100.0	17.3	96.2
0.90	H-O	5.7	67.9	50.1	47.6	3.9	14.7	4.4	34.2	75.8	100.0	100.0	18.7	94.8
	SH	5.7	70.9	49.1	46.6	4.2	16.7	5.5	44.2	86.1	100.0	100.0	80.2	22.4

Abbreviations: G. cor = Genetic correlation of SNP effects on incidence and prognosis; MSE = Mean square error; G_1 & G_2 = All SNPs affecting incidence; MAB = mean of the maximum absolute bias over simulations ($\times 10^{-3}$); FWE = family-wise type-1 error (%); HE = Type-1 error of the SNP with highest error for the unadjusted estimator (%); IP = Power of the SNP with greatest increase in power between the unadjusted and adjusted estimators; DP = Power of the SNP with greatest decrease in power between the unadjusted and adjusted estimators; H-O = the 'Hedges-Olkin' estimator; SH = 'Slope-Hunter' estimator.

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6 Figures

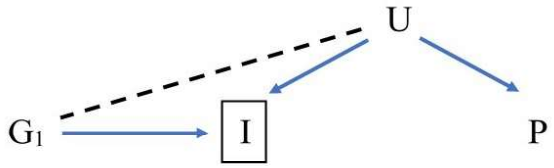

Figure 1: Directed acyclic graph for association of a SNP G_1 with a prognosis trait P conditional on an incidence trait I. U is a composite variable including all common causes of I and P, including polygenic effects as well as non-genetic factors. Conditioning on I induces the association between G_1 and U, shown by the dashed line, leading to biased association between G_1 and P via the path $G_1 - U \rightarrow P$. The association of G_1 with the prognosis P when conditioning on incidence is entirely due to the index event bias

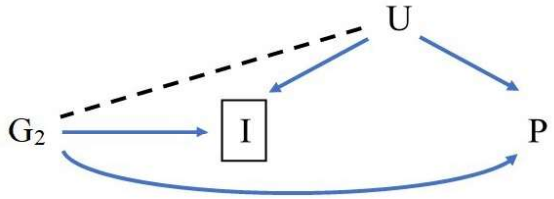

Figure 2: Directed acyclic graph for association of a SNP G_2 with a prognosis trait P conditional on an incidence trait I. U is a composite variable including all common causes of I and P, including polygenic effects as well as non-genetic factors. Conditioning on I induces the association between G_2 and U, shown by the dashed line, leading to association between G_2 and P via the path $G_2 - U \rightarrow P$, in addition to the direct effect $G_2 \rightarrow P$.

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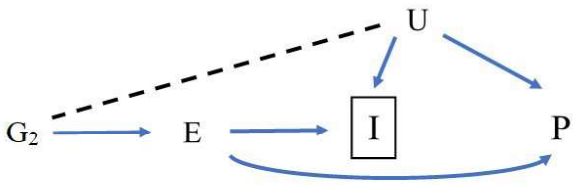

Figure 3: Directed acyclic graph for association of a SNP G_2 with a prognosis trait P conditional on an incidence trait I. U is a composite variable including all common causes of I and P, including polygenic effects as well as non-genetic factors. There is a shared pathway for both incidence and prognosis via an exposure E leading to correlation between effects on prognosis and effects on incidence. Conditioning on I induces the association between G_2 and U, shown by the dashed line, leading to association between G_2 and P via the path $G_2 - U \rightarrow P$, in addition to the effect $G_2 - E \rightarrow P$.

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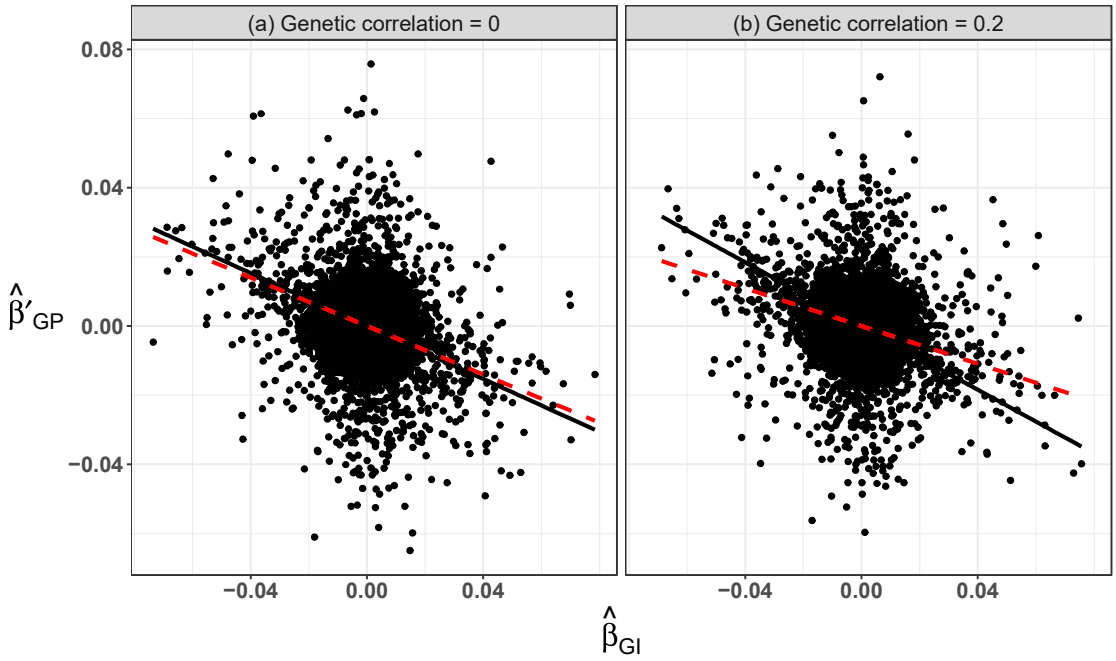

Figure 4: Scatter plots for estimates of SNP-incidence associations, $\hat{\beta}_{GI}$, and conditional estimates, $\hat{\beta}'_{GP}$, simulated from dataset of 20,000 individuals for 10,000 independent SNPs, with: (a) no genetic correlation between SNP effects on incidence and prognosis; (b) correlated genetic effects (correlation coefficient = 0.2). Five-percent of SNPs have effects on incidence only, 5% on prognosis only, and 5% on both. Heritability of incidence and prognosis is 50% and non-genetic common factors of explain 40% of variation in both incidence and prognosis. These simulations induced index event bias due to confounders that explain 40% and 60% of variation in prognosis in (a) and (b) respectively. The true confounding effects are represented by slopes of the black solid lines, (a) -0.38 and (b) -0.46, while the estimated correction factors using Dudbridge et. al. (2019) method are represented by slopes of the red dashed lines, (a) -0.35 and (b) -0.27. This figure illustrates potential inadequate correction using the Dudbridge et. al. (2019) method when the 'InCLUDE' assumption (Index Coefficient Linearly Uncorrelated with Direct Effect) is violated.

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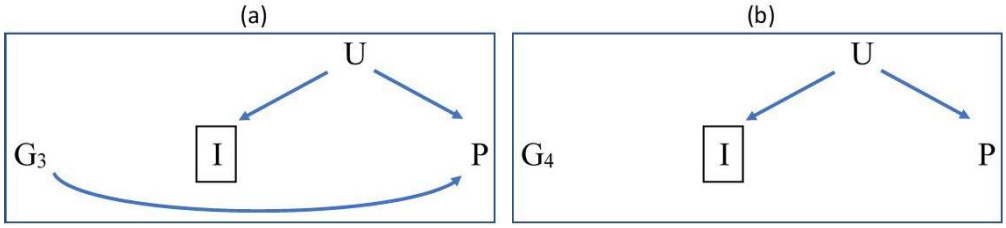

Figure 5: Directed acyclic graph for association of: (a) a SNP G_3 with a direct effect on a prognosis trait P, with no effect on incidence I; (b) a SNP G_4 with effect on neither I nor P, conditional on an incidence trait I. U is a composite variable including all common causes of I and P, including polygenic effects as well as non-genetic factors. Conditioning on I does not induce biased association between either G_3 or G_4 and P as no association between G_3 or G_4 and U are produced since either SNP does not affect I.

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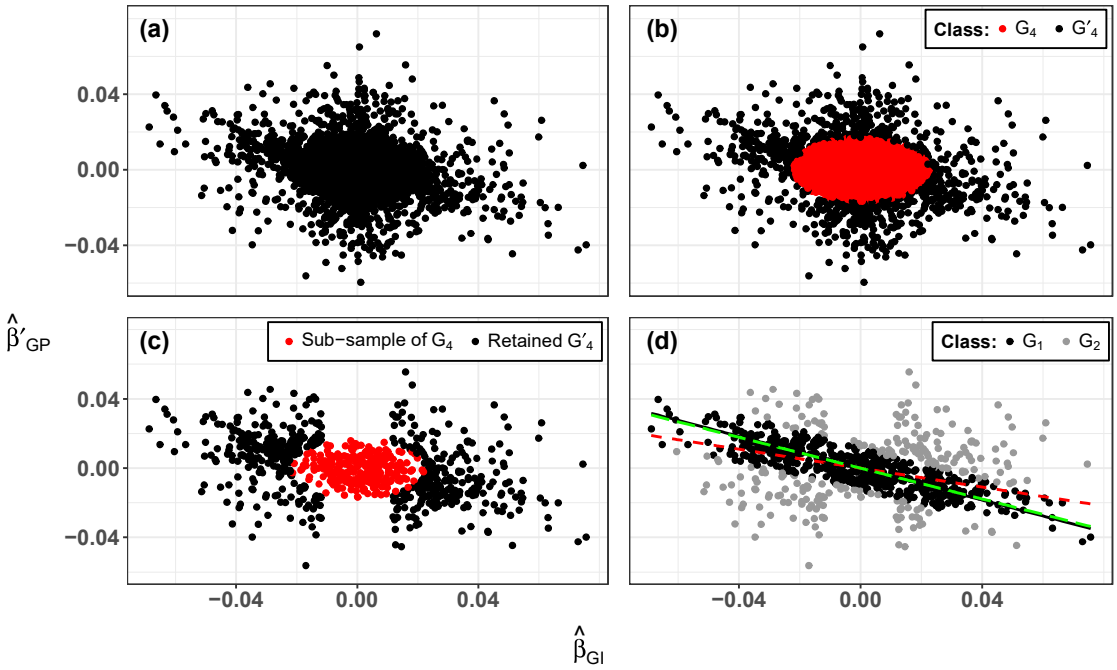

Figure 6: A graphical illustration for phases of the Slope-Hunter approach: (a) inputs of SNP-incidence associations, $\hat{\beta}_{GI}$, and conditional estimates, $\hat{\beta}'_{GP}$, shown in Figure 4(b), that were simulated with correlated genetic effects (correlation coefficient = 0.2) on incidence and prognosis; (b) Slope-Hunter identifies variants affecting neither incidence nor prognosis, i.e. the class of G_4 SNPs; (c) a random sub-sample of G_4 SNPs is retained in the analysis, whereas the remaining variants in this group and the variants affecting prognosis only, i.e. the class of G_3 SNPs, are excluded. The latter is identified using a p-value threshold for SNP-incidence associations; (d) The class of variants affecting incidence only, G_1, is identified and an estimate of its linear regression slope (represented by green long-dashed line, slope = -0.445) is obtained to correct for the index event bias of all SNPs. The true confounding effect is represented by the black solid line's slope, -0.460, whereas the estimated correction factor using Dudbridge et. al. (2019) method is represented by the red dashed line's slope, -0.273.

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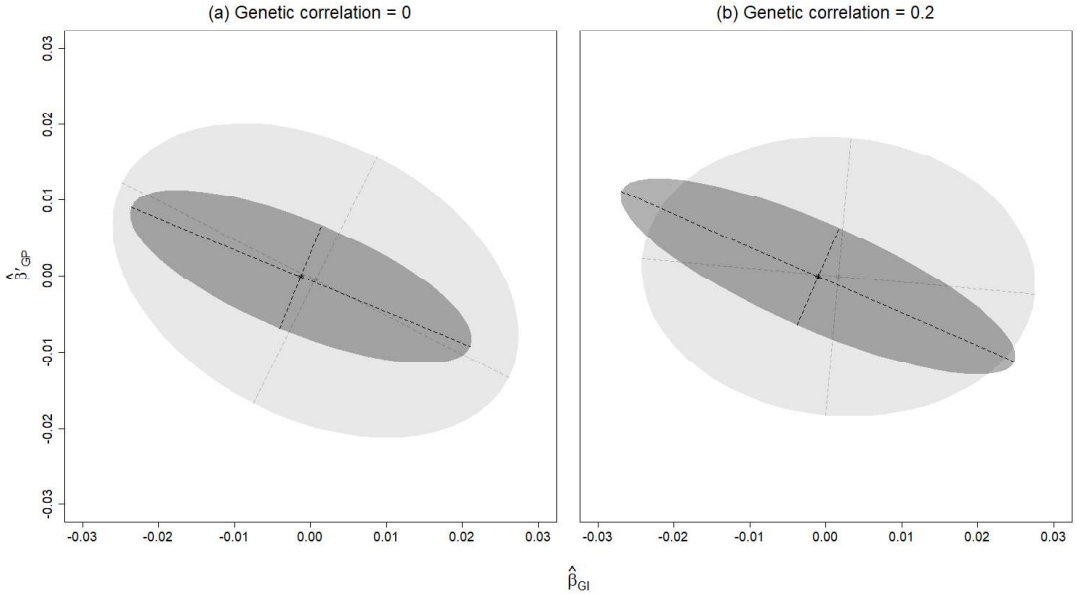

Figure 7: Illustration of mixture components (clusters) estimated by the Slope-Hunter method for the class of variants affecting incidence only (darker colour) and the calss of variants affecting both incidence and prognosis (lighter colour) based on values of SNP-incidence associations, $\hat{\beta}_{GI}$, and conditional estimates, $\hat{\beta}'_{GP}$ simulated from dataset of 20,000 individuals for 10,000 independent SNPs, with: (a) no genetic correlation between SNP effects on incidence and prognosis; (b) correlated genetic effects (correlation coefficient = 0.2). Each cluster is ellipsoidal with geometric features: area (coloured); shape (of the estimated ellipse); orientation (illustrated by orthogonal dashed lines, i.e. axes of the ellipse), that are determined by its covariance matrix [15, 16]

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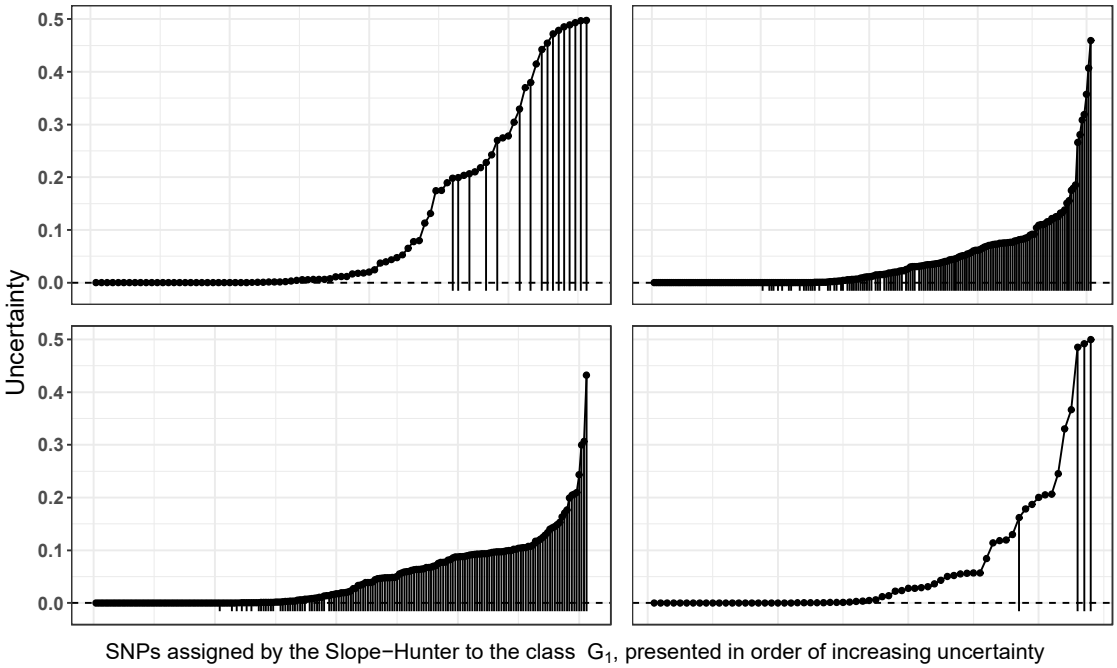

Figure 8: Uncertainty plot for SNPs identified by the Slope-Hunter method as the variants affecting incidence only from four simulations of scenario 4 in which 1% of SNPs have effects on incidence only and 9% on both incidence and prognosis with a correlation of -0.9, explaining 45% and 5% respectively of variation in incidence. The vertical lines indicate misclassified SNPs.

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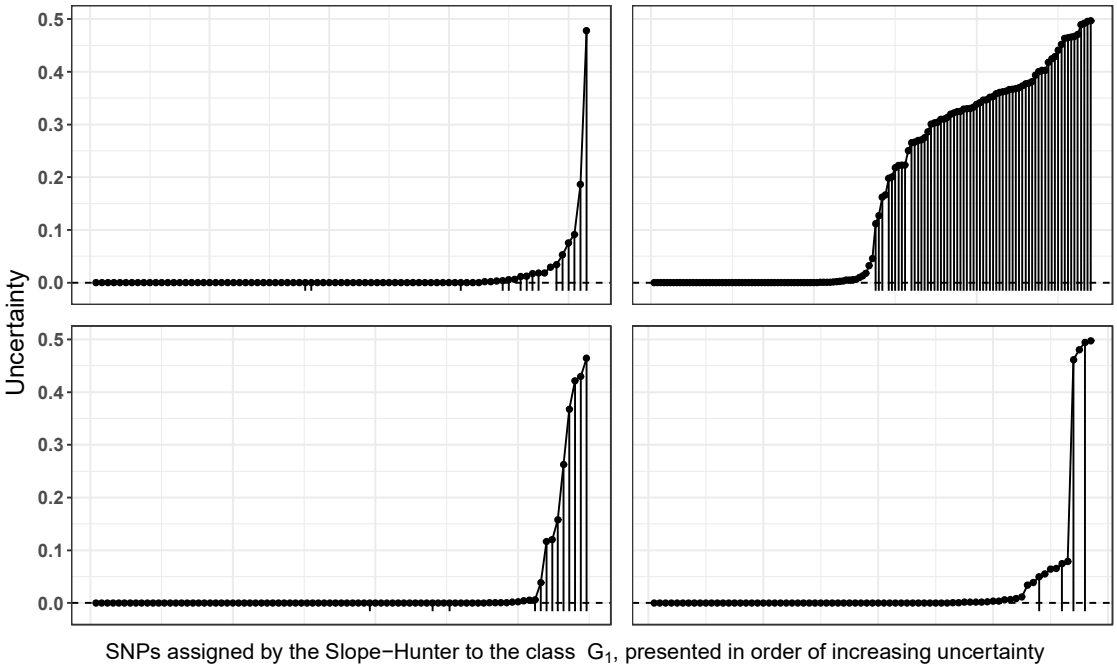

Figure 9: Uncertainty plot for SNPs identified by the Slope-Hunter method as the variants affecting incidence only from four simulations of scenario 4 in which 1% of SNPs have effects on incidence only and 9% on both incidence and prognosis with a correlation of 0.9, explaining 45% and 5% respectively of variation in incidence. The vertical lines indicate misclassified SNPs.