Supplementary Materials

Estimating and testing direct genetic effects in directed acyclic graphs using estimating equations

Stefan Konigorski, Yuan Wang, Candemir Cigsar, Yildiz E. Yilmaz

**Supplementary Text**

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# Supplementary Text 1 – Details on CIEE for the analysis of time-to-event primary traits

For an illustration of CIEE under the AFT model, we consider the log-normal model with in equation (10). To obtain the adjusted phenotype in equation (12), the true log survival times in equation (11) are derived as

where , is the probability density function of Y given K, X, L, is the cumulative distribution function of given K, X, L under the model in equation (10), is the standard normal probability density function, and is the standard normal cumulative distribution function.

The functions with , in the estimating equations in equation (14) are derived as follows. is the log-likelihood function under the model in (10). Hence, for right censored time-to-event data it is obtained as

is the log-likelihood function under the model in equation (13) given that is known. Hence, it is

# Supplementary Text 2 – Details on the data generation model in the simulation study

For the simulation study, data was generated from the models in Figure 2 with effect sizes as described in Supplementary Table 1 based on the following set-up, for n independent observations.

The generation of genotypes X contains the assumption of additive genetic effects. Alternatively, other genetic models such as a dominant genetic model with could be used.

For the data generation under the AFT setting, the following additional steps were followed. First, we generated the time-to-event phenotype for each individual . Then, we generated the right-censoring time from the uniform distribution with parameters so that on average, of the individuals were censored as pre-specified:

The observed time-to-event data is then the minimum of and , , and the censoring indicator is .

The parameters a, b were chosen as shown in the following table:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Investigation** | **Scenario** |  |  |  |  |
| Type I error | 1 | 10%; 30%; 50% | 0 | 0.3; 0.2; 0 | 14.75; 4.55; 2.48 |
| 2 | 10%; 30%; 50% | 0 | 0.3; 0.2; 0 | 14.90; 4.58; 2.49 |
| 3 | 10%; 30%; 50% | 0 | 0.3; 0.2; 0 | 15.00; 4.58; 2.49 |
| 4 | 10%; 30%; 50% | 0 | 0.3; 0.2; 0 | 15.80; 4.70; 2.50 |
| 5 | 10%; 30%; 50% | 0 | 0.3; 0.2; 0 | 14.25; 4.40; 2.42 |
| Power | 1 | 30% | 0.1; 0.2 | 0.2 | 4.75; 5.00 |
| 2 | 30% | 0.1; 0.2 | 0.2 | 4.81; 5.05 |
| 3 | 30% | 0.1; 0.2 | 0.2 | 4.80; 5.05 |
| 4 | 30% | 0.1; 0.2 | 0.2 | 4.94; 5.17 |
| 5 | 30% | 0.1; 0.2 | 0.2 | 4.60; 4.85 |

Overview of the parameters in the Uniform distribution used to generate censoring times in the simulation study.

# Supplementary Text 3 – Discussion of the sequential G-estimation approach for time-to-event traits

Lipman et al. (2011) proposed a method to detect direct genetic effects on a target time-to-event phenotype in the presence of genetic associations with an intermediate phenotype that affects the target time-to-event phenotype. The method is based on the methodology introduced in Vansteelandt et al. (2009) where the target phenotype was a completely observed quantitative variable, which was modeled by a linear regression model. To estimate and test the direct effect of a genetic marker on the target phenotype, Vansteelandt et al. (2009) presented a two-stage method which includes an adjustment stage to remove the effect of an intermediate phenotype on the target phenotype. This method uses the principle of the sequential G-estimation (Goetgeluk, Vansteelandt, & Goetghebeur, 2008) for making inference on direct effects in linear models. In the first stage, the effect of the intermediate phenotype on the target phenotype is estimated and an adjusted target phenotype is obtained by removing the effect of the intermediate phenotype. In the second stage, inference on the direct effect of the genetic marker on the target phenotype is obtained by regressing the adjusted target phenotype on the genetic marker.

The two-stage method works effectively under a linear regression model with a completely observed outcome. The goal of Lipman et al. (2011) was to extend the two-stage method to the setting in which the target phenotype is a time-to-event variable and subject to censoring. They considered proportional hazards regression and accelerated failure time models of a time-to-event phenotype, and proposed a new adjustment method to remove the effect of the intermediate phenotype on the target time-to-event phenotype. They obtained the adjusted survival times by subtracting the mean of observed survival times and the so-called “partial” Deviance residuals from the observed survival times (equation (4) in Lipman et al., 2011). In the following, we show that this adjustment method does not remove the effect of the intermediate phenotype on the target time-to-event phenotype.

We assessed whether the effect of the intermediate phenotype is removed from the defined adjusted target time-to-event phenotype given in equation (4) of Lipman et al. (2011). To this end, we conducted a Monte Carlo simulation study. The design of our simulation study is similar to that of Lipman et al. (2011), but to be able to explain our points easily, we considered the following simpler causal diagram:

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DAG considered in the simulation study described in Supplementary Text 3.

We generated 1,000 replicates, each of sample size 1,000. The genotype X was sampled from the Bernoulli distribution with mean , representing a dominant genetic effects model, and the intermediate phenotype K from the normal distribution with mean and variance 1. Furthermore, we generated the target phenotype (i.e. survival times T) from the proportional hazards regression model with hazard function , where is the Weibull baseline hazard function with a unit scale parameter and shape parameter 1.0 or 1.5. We considered a simple scenario, where survival times are all observed and there is no censoring. This simple setup allowed us a better examination of whether the adjustment method of Lipman et al. (2011) works.

After applying the adjustment procedure in Lipman et al. (2011) to each replication sample, we investigated whether the intermediate phenotype K has any effect on the adjusted target phenotype . To be able to assess the effect of K on , one option is to fit a plausible parametric conditional model for given K and X (i.e. ). Based on the assumption that the effect of K on is removed, Lipman et al. (2011) suggested to use a standard linear regression model of in their equation (5) to estimate the direct effect of X on T. However, there is no discussion why this model was used. The appropriateness of a linear regression model of is in question. Nevertheless, we first considered the linear regression model of as it was suggested. Under this model assumption, if the adjustment method successfully removes the effect of the intermediate phenotype K on T, the coefficient of K will be zero in the linear regression model of . The following table displays the empirical means and standard deviations of least square estimates of the coefficient of K over 1,000 replicates, which shows that the effect of K on the adjusted target phenotype has not been removed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  |  |
|  | 0.2 | 0.2 | -0.17 | 0.03 |
| 1.0 | 0.2 | -0.15 | 0.03 |
| 0.2 | 1.0 | -0.82 | 0.12 |
| 1.0 | 1.0 | -0.79 | 0.13 |
|  | 0.2 | 0.2 | -0.11 | 0.02 |
| 1.0 | 0.2 | -0.10 | 0.02 |
|  |  | -0.47 | 0.04 |
|  |  | -0.44 | 0.04 |

Empirical mean and empirical standard deviation (sd) of under the model with and .

However, when we checked the linear regression assumptions, we observed that the linearity and homoscedasticity assumptions were violated. We also noticed that it is difficult to confirm a plausible distribution and a plausible model for . Therefore, we assessed the association between the adjusted phenotype and the intermediate phenotype K using a nonparametric association measure instead of assuming a parametric form to model the causal relationship. We used a rank-based association measure, Kendall’s tau (), to assess the association between and K. The table below shows the proportion of p-values that are below the nominal value of 0.05 for testing the null hypothesis that K is not associated with (i.e. ) for each stratum of X. We observed that there is still a strong association between the adjusted target phenotype and the intermediate phenotype K and the association becomes stronger as the effect of K on T, , increases.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  | |
|  | 0.2 | 0.2 | 0.55 | 0.13 |
| 1.0 | 0.2 | 0.52 | 0.09 |
| 0.2 | 1.0 | 1 | 0.76 |
| 1.0 | 1.0 | 1 | 0.44 |
|  | 0.2 | 0.2 | 0.39 | 0.12 |
| 1.0 | 0.2 | 0.36 | 0.09 |
|  |  | 1 | 0.85 |
|  |  | 1 | 0.63 |

Proportion of rejected null hypothesis that there is no association between the intermediate phenotype and the adjusted target time-to-event phenotype. The proportions are reported for each stratum of X, e.g. for X=0 and X=1.

Contrary to what is proposed in Lipman et al. (2011), we showed here that the effect of the intermediate phenotype is not removed from the adjusted target time-to-event phenotype. Hence, the direct genetic effect on the target time-to-event phenotype cannot be estimated using the proposed approach under the causal directed acyclic graph. In addition, there is no discussion on whether is adjusted for censoring and how a standard linear regression model without considering censoring (as in equation (5) in Lipman et al., 2011) could be used to estimate the direct effect of a genetic marker X on the time-to-event variable T. Even when there is no censoring, modeling the adjusted target time-to-event phenotype by using a standard linear regression model of as in equation (5) in Lipman et al. (2011) is not a valid approach, as is using the test statistic for testing the absence of a direct genetic effect on the target time-to-event phenotype.

# Supplementary Tables

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Setting** | **Investigation** | **Scenario** |  |  |  |  |  |  |  |  |  |  |
| LM | Type I error | 1 | - | 0.05; 0.1; 0.2; 0.4 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0.3 | 0 |
| 2 | - | 0.05; 0.1; 0.2; 0.4 | 0 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0 |
| 3 | - | 0.05; 0.1; 0.2; 0.4 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0 |
| 4 | - | 0.05; 0.1; 0.2; 0.4 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0.3 | 0 |
| 5 | - | 0.05; 0.1; 0.2; 0.4 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0 | 0 |
| 6 |  | 0.05; 0.1; 0.2; 0.4 | 0 | 0 | 0.2 | 0.3 | 0.3 | 0 | 0.3 | 0 |
| 7 | - | 0.05; 0.1; 0.2; 0.4 | 0.5 | 1 | 1 | 0.5 | 0 | 0.5 | 1 | 0 |
| Power | 1 | - | 0.2 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 2 | - | 0.2 | 0 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 3 | - | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 4 | - | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0.3 | 0.1; 0.2 |
| 5 | - | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0 | 0.1; 0.2 |
| AFT | Type I error | 1 | 10%; 30%; 50% | 0.2 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0.3 | 0 |
| 2 | 10%; 30%; 50% | 0.2 | 0 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0 |
| 3 | 10%; 30%; 50% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0 |
| 4 | 10%; 30%; 50% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0.3 | 0 |
| 5 | 10%; 30%; 50% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0 | 0 |
| Power | 1 | 30% | 0.2 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 2 | 30% | 0.2 | 0 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 3 | 30% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0 | 0.3 | 0.1; 0.2 |
| 4 | 30% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0.3 | 0.1; 0.2 |
| 5 | 30% | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0 | 0.3 | 0 | 0.1; 0.2 |

Supplementary Table 1. Overview of the scenarios in the simulation study.

In all scenarios, data was generated for individuals and replicates, with , .

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Scenario** |  |  |  |  |  |  |
| 1 |  | (0.10) | (0.10) | (0.10) | (0.10) | (0.10) |
|  | (0.07) | (0.07) | (0.08) | (0.07) | (0.07) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 2 |  | (0.10) | (0.10) | (0.10) | (0.10) | (0.10) |
|  | (0.07) | (0.08) | (0.08) | (0.07) | (0.08) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 3 |  | (0.10) | (0.10) | (0.10) | (0.10) | (0.10) |
|  | (0.07) | (0.07) | (0.08) | (0.07) | (0.08) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 4 |  | (0.11) | (0.11) | (0.11) | (0.11) | (0.11) |
|  | (0.08) | (0.08) | (0.08) | (0.07) | (0.08) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 5 |  | (0.11) | (0.11) | (0.11) | (0.11) | (0.11) |
|  | (0.08) | (0.08) | (0.08) | (0.07) | (0.08) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 6 |  | (0.11) | (0.11) | (0.10) | (0.10) | (0.11) |
|  | (0.08) | (0.08) | (0.08) | (0.07) | (0.08) |
|  | (0.06) | (0.06) | (0.06) | (0.06) | (0.06) |
|  | (0.05) | (0.05) | (0.05) | (0.05) | (0.05) |
| 7 |  | (0.13) | (0.13) | (0.12) | (0.11) | (0.12) |
|  | (0.10) | (0.10) | (0.09) | (0.08) | (0.09) |
|  | (0.08) | (0.08) | (0.08) | (0.06) | (0.08) |
|  | (0.07) | (0.07) | (0.07) | (0.05) | (0.07) |

Supplementary Table 2. Empirical mean of the direct effect estimates and their standard error estimates obtained through different methods under the null model of a quantitative primary phenotype.

CIEE is the proposed method using estimating equations; BS is CIEE using nonparametric bootstrap standard errors; MR is multiple regression; RR is residual regression; SEM is structural equation modeling. is the MAF of the marker X. Shown are mean coefficient and standard error estimates of the direct genetic effect over the replicates. Coefficient estimates obtained from BS and sequential G-estimation (Vansteelandt et al., 2009) are identical to those of CIEE and not shown. Data was generated for individuals. The scenarios when the amount of bias of was larger than 0.01 are highlighted in red.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Scenario** |  |  |  |  |  |  |
| 1 |  |  |  |  |  |  |
|  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |
|  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |
|  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |
|  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |
|  |  |  |  |  |  |

Supplementary Table 3. Empirical mean of the direct effect estimates and their standard error estimates obtained through different methods under the alternative hypothesis models of a quantitative primary phenotype.

CIEE is the proposed method using estimating equations; BS is CIEE using nonparametric bootstrap standard errors; MR is multiple regression; RR is residual regression; SEM is structural equation modeling. Shown are mean coefficient and standard error estimates of the direct genetic effect over the replicates. Coefficient estimates from BS and sequential G-estimation (Vansteelandt et al., 2009) are identical to those of CIEE and not shown. Data was generated for individuals for a genetic marker with . The scenarios where the amount of bias of was larger or equal to 0.01 are highlighted in red.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Scenario** | **Censoring** |  |  |  |  |
| 1 |  | (0.06) | (0.06) | 0.20 | (0.06) |
|  | (0.06) | (0.06) | 0.15 | (0.06) |
|  | (0.07) | (0.07) | 0.11 | (0.07) |
| 2 |  | (0.06) | (0.06) | 0.25 | (0.06) |
|  | (0.06) | (0.06) | 0.20 | (0.06) |
|  | (0.07) | (0.07) | 0.15 | (0.07) |
| 3 |  | (0.06) | (0.06) | 0.25 | (0.06) |
|  | (0.06) | (0.06) | 0.19 | (0.06) |
|  | (0.07) | (0.07) | 0.15 | (0.07) |
| 4 |  | (0.06) | (0.06) | 0.25 | (0.06) |
|  | (0.06) | (0.06) | 0.19 | (0.06) |
|  | (0.07) | (0.07) | 0.14 | (0.07) |
| 5 |  | (0.06) | (0.06) |  | (0.06) |
|  | (0.06) | (0.06) |  | (0.06) |
|  | (0.07) | (0.07) |  | (0.07) |

Supplementary Table 4. Empirical mean of the direct effect estimates and their standard error estimates obtained through different methods under the null model of a time-to-event primary phenotype.

CIEE is the proposed method using estimating equations; BS is CIEE using nonparametric bootstrap standard errors; G-EST is the sequential G-estimation approach (Lipman et al., 2011); MR is multiple regression. Shown are mean coefficient and standard error estimates of the direct genetic effect over the replicates. Coefficient estimates from BS are identical to those of CIEE and not shown. Data was generated for individuals for a genetic marker with and different censoring rates. The scenarios where the amount of bias of was larger than 0.01 are highlighted in red.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Scenario** |  |  |  |  |  |
| 1 |  |  |  |  |  |
|  |  |  |  |  |
| 2 |  |  |  |  |  |
|  |  |  |  |  |
| 3 |  |  |  |  |  |
|  |  |  |  |  |
| 4 |  |  |  |  |  |
|  |  |  |  |  |
| 5 |  |  |  |  |  |
|  |  |  |  |  |

Supplementary Table 5. Empirical mean of the direct effect estimates and their standard error estimates obtained through different methods under the alternative hypothesis models of a time-to-event primary phenotype.

CIEE is the proposed method using estimating equations; BS is CIEE using nonparametric bootstrap standard errors; G-EST is the sequential G-estimation approach (Lipman et al., 2011); MR is multiple regression. Shown are mean coefficient and standard error estimates of the direct genetic effect over the replicates. Coefficient estimates obtained through BS are identical to those of CIEE and not shown. Data was generated for individuals for a genetic marker with , with 30% censoring. The scenarios where the amount of bias of was larger than 0.01 are highlighted in red.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Distribution of Y|X,K,L,U** |  |  |  | **Type I error** |
|  |  | 0.08 | 0.08 | 4.97% |
|  |  | 0.09 | 0.09 | 5.17% |
|  |  | 0.11 | 0.11 | 4.92% |
|  |  | 0.16 | 0.16 | 4.87% |

Supplementary Table 6. Results of CIEE under the null hypothesis when the distribution of the quantitative primary phenotype is misspecified.

Shown are mean estimates of the direct genetic effect, , mean standard error estimates of the estimated direct genetic effect , standard deviation of the direct genetic effect estimates, , and empirical type I error estimates obtained through CIEE under the null model of a quantitative primary phenotype. Data was generated for individuals and replicates for a genetic marker with , from scenario 7 under the null hypothesis. The distribution of the primary phenotype given covariates in the data generation was chosen to be from a standard normal (cf. Table 1, Supplementary Table 2),, or log standard normal distribution.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Scenario** |  |  |  |  |
| 1 | 0.1 | 38.30% | 38.15% | 38.15% |
| 0.2 | 91.14% | 91.10% | 91.30% |
| 2 | 0.1 | 38.24% | 38.02% | 37.50% |
| 0.2 | 90.85% | 90.86% | 91.04% |
| 3 | 0.1 | 38.15% | 37.94% | 37.54% |
| 0.2 | 90.79% | 90.74% | 90.57% |
| 4 | 0.1 | 35.37% | 35.10% | 25.85% |
| 0.2 | 88.23% | 88.14% | 82.04% |
| 5 | 0.1 | 35.46% | 35.25% | 25.90% |
| 0.2 | 88.73% | 88.52% | 83.49% |

Supplementary Table 7. Power estimates under the alternative hypothesis models of a time-to-event primary phenotype.

Data was generated for individuals and replicates for a genetic marker with , with 30% censoring. CIEE is the proposed method using estimating equations; BS is CIEE using nonparametric bootstrap standard errors; and MR is the multiple log-linear censored regression approach. Highlighted in red are those scenarios where the methods yielded inflated type I error.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNP** |  |  |  | | | **95% CI of** | | |  | | |  | | |
|  |  |  |  |  |  |  |  |  |  |  |  |
| rs62117661 | 0.09 | *KLK12* | 0.26  (0.06) | 0.18  (0.04) | 0.18  (0.04) | (0.14; 0.37) | (0.10; 0.26) | (0.10; 0.26) |  |  |  | 0.55 | 0.46 | 1 |
| rs1972785 | 0.31 | *ZSCAN5A* | -0.09  (0.03) | -0.09  (0.02) | -0.09  (0.02) | (-0.14; -0.03) | (-0.14; -0.05) | (-0.14; -0.05) |  |  |  | 1 | 0.94 | 1 |
| rs62117661 | 0.09 | *KLK11* | 0.25  (0.06) | 0.18  (0.04) | 0.18  (0.04) | (0.14; 0.37) | (0.09; 0.26) | (0.10; 0.26) |  |  |  | 1 | 0.94 | 1 |
| rs10415616 | 0.35 | *ZSCAN5A* | -0.07  (0.03) | -0.09  (0.02) | -0.09  (0.02) | (-0.12; -0.02) | (-0.13; -0.05) | (-0.13; -0.05) |  |  |  | 1 | 0.94 | 1 |
| rs7248173 | 0.31 | *ZSCAN5A* | -0.09  (0.03) | -0.09  (0.02) | -0.09  (0.02) | (-0.14; -0.03) | (-0.14; -0.05) | (-0.14; -0.05) |  |  |  | 1 | 1 | 1 |

Supplementary Table 8. Top 5 SNPs with the smallest p-values in the GAW19 genetic association analysis using multiple regression.

Top 5 SNPs with the strongest association with systolic blood pressure obtained under the traditional multiple regression genetic association analysis of 113,890 SNPs on chromosome 19, with the corresponding gene (expression) as intermediate phenotype. SNPs are described by their rs identification numbers. Point estimates, standard error estimates, approximate 95% confidence intervals (CI), raw p-values and Bonferroni-corrected p-values of CIEE and the multiple regression approaches MR1 and MR2 are presented. MAF is the observed MAF of the SNP.

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