Appendix A. Mathematical framework of cov-LDSC

Here, we will first provide a derivation of standard LD score regression that differs somewhat from published derivations, and in particular gives a mathematical interpretation for the value of the intercept. Then we will extend this derivation to cov-LDSC.

A.1 Review of LD score regression without covariates

A.1.1 Summary statistics without covariates

We begin by describing the input data to LD score regression, which is the output of a standard GWAS. In a standard GWAS of a quantitative trait, a marginal linear model is fit for each SNP j. Let Y denote the $N \times 1$ vector of phenotypes and X_j denote the $N \times 1$ vector of genotypes for SNP j, centered to mean zero. In the absence of covariates, we typically fit the model

$$Y = X_j \beta_j^{(marg)} + \varepsilon^{(marg)}, \tag{A1}$$

where $\beta_j^{(marg)}$ is the marginal effect size of SNP j and $\varepsilon^{(marg)} \sim N(0, \sigma^2{}_{(marg)} I)$.

The F-statistic, which at GWAS sample sizes is approximately chi-square distributed under the null and often referred to as the chi-square statistic, is equal to

$$\chi_j^2 = \left(\hat{\beta}_j^{(marg)}\right)^2 / \hat{s}_j^2 \tag{A2}$$

where

$$\hat{\beta}_j^{(marg)} = \frac{X_j^T Y}{X_j^T X_j},$$

and

$$\hat{s}_j^2 = \frac{\hat{\sigma}^2_{(marg)}}{X_j^T X_j},$$

where $\hat{\sigma}^2_{(marg)}$ is an estimate of $\sigma^2{}_{(marg)}$ that, if $\hat{\beta}_j^{(marg)}$ is small, satisfies

$$\hat{\sigma}^2_{(marg)} \approx \frac{1}{N} Y^T Y.$$

We will assume that $\beta_j^{(marg)}$ and its estimate $\hat{\beta}_j^{(marg)}$ are indeed small, so that this is a valid approximation.

Let $V(X_j) = X_j^T X_j / N$ and $V(Y) = Y^T Y / N$ be the empirical variances of X_j and Y, and let $\tilde{X}_j = X_j / \sqrt{V(X_j)}$, and $\tilde{Y} = Y / \sqrt{V(Y)}$ be X_j and Y, normalized to empirical variance one. Note that when X_j and Y are random, so are $V(X_j), V(Y), \tilde{X}_j$, and $\tilde{Y}$. Note also that $\tilde{X}_j^T \tilde{X}_j = \tilde{Y}^T \tilde{Y} = N$, deterministically. We can now simplify the expression

for χ_j^2:

$$\chi_j^2 \approx \frac{1}{N}(\tilde{X}_j^T \tilde{Y})^2. \tag{A3}$$

We will assume that we have as input χ_j^2 for a genome-wide set of SNPs j.

A.1.2 The polygenic model

In LD score regression, we take these chi-square statistics as input, and we derive their expectation under a standard polygenic model. Specifically, instead of the marginal model used in GWAS, LD score regression is based on a joint model with random SNP effect sizes:

$$Y = X\beta + \varepsilon,$$

where Y is the phenotype vector, $X = (X_1 \ldots X_M)$ is the $N \times M$ genotype matrix, $\varepsilon \sim \mathcal{N}(0, \sigma_\varepsilon^2 I)$, and β is the $M \times 1$ vector of joint effect sizes. Let $\tilde{\beta}_j = \beta_j \sqrt{V(X_j)}$, and note that $X\beta = \tilde{X}\tilde{\beta}$. We will model $\tilde{\beta}_j$ as random with mean zero, independent of each other and of ε. Here, we will perform derivations in which $Var(\tilde{\beta}_j) = \sigma_{\tilde{\beta}}^2$; these derivations extend easily to the case in which $Var(\tilde{\beta}_j)$ depends on functional annotations. We don't specify a distribution for $\tilde{\beta}$.

In LD score regression, we derive the expectation of χ_j^2 under this polygenic model, and we use the resulting equation to estimate parameters such as $\sigma_{\tilde{\beta}}^2$. Because X is not observed, we ultimately treat it as random. Here, we will derive $E[\chi_j^2]$ by first deriving $E[\chi_j^2|X]$ and then using the law of total expectation to remove the conditioning on X.

A.1.3 Deriving the expression for $E[\chi_j^2|X]$

Before deriving the expression for $E[\chi_j^2|X]$, we will first derive the expected empirical variance of Y, where the variance is over the random individuals in our GWAS and the expectation is over random β and ε, conditional on X.

$$
\begin{aligned}
E[V(Y)|X] &= \frac{1}{N}E\left[(X\beta+\varepsilon)^T(X\beta+\varepsilon)|X\right] \\
&= \frac{1}{N}E\left[\left(\tilde{X}\tilde{\beta}+\varepsilon\right)^T\left(\tilde{X}\tilde{\beta}+\varepsilon\right)|X\right] \\
&= \frac{1}{N}E\left[\tilde{\beta}^T\tilde{X}^T\tilde{X}\tilde{\beta}|X\right]+\frac{1}{N}E\left[\varepsilon^T\varepsilon\right] \\
&= \frac{1}{N}\sum_{j,k}E\left[\tilde{\beta}_j(\tilde{X}^T\tilde{X})_{j,k}\tilde{\beta}_k|X\right]+\sigma_\varepsilon^2 \\
&= \frac{1}{N}\sum_{j\neq k}E\left[\tilde{\beta}_j\right]E\left[\tilde{\beta}_k\right](\tilde{X}^T\tilde{X})_{j,k}+\frac{1}{N}\sum_{j}E\left[\tilde{\beta}_j^2\right](\tilde{X}^T\tilde{X})_{j,j}+\sigma_\varepsilon^2 \\
&= 0+\frac{1}{N}\sum_{j}\sigma_{\tilde{\beta}}^2(\tilde{X}^T\tilde{X})_{j,j}+\sigma_\varepsilon^2 \\
&= M\sigma_{\tilde{\beta}}^2+\sigma_\varepsilon^2
\end{aligned}
$$

We will let h_g^2 denote $M\sigma_{\tilde{\beta}}^2/E[V(Y)|X]$, noting that definitions of heritability depend on the model on which they are based, and so h_g^2 as used here is a different value than in a model in which β is fixed.

It will also be useful to have

$$
\begin{aligned}
E\left[\left(\tilde{X}_j^T\varepsilon\right)^2|X\right] &= E\left[\tilde{X}_j^T\varepsilon\varepsilon^T\tilde{X}_j|X\right] \\
&= \tilde{X}_j^T E\left[\varepsilon\varepsilon^T\right]\tilde{X}_j \\
&= \sigma_\varepsilon^2\tilde{X}_j^T\tilde{X}_j \\
&= N\sigma_\varepsilon^2
\end{aligned}
$$

We can now derive the expected chi-square statistic:

$$\begin{aligned}
E[\chi_j^2|X] &= E\left[\frac{1}{N}\left(\tilde{X}_j^T\tilde{Y}\right)^2|X\right] \\
&= E\left[\frac{1}{NV(Y)}\left(\tilde{X}_j^T\left(X\beta+\varepsilon\right)\right)^2|X\right] \\
&\approx \frac{1}{NE[V(Y)|X]}E\left[\left(\tilde{X}_j^T\left(X\beta+\varepsilon\right)\right)^2|X\right] \\
&= \frac{1}{NE[V(Y)|X]}E\left[\left(\tilde{X}_j^T\left(\tilde{X}\tilde{\beta}+\varepsilon\right)\right)^2|X\right] \\
&= \frac{1}{NE[V(Y)|X]}E\left[\left(\sum_k \tilde{X}_j^T\tilde{X}_k\tilde{\beta}_k+\tilde{X}_j^T\varepsilon\right)^2|X\right] \\
&= \frac{N}{E[V(Y)|X]}\sum_k\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2 E[\tilde{\beta}_k^2]+\frac{1}{NE[V(Y)|X]}E\left[\left(\tilde{X}_j^T\varepsilon\right)^2|X\right] \\
&= \frac{N\sigma_{\tilde{\beta}}^2}{E[V(Y)|X]}\sum_k\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2+\frac{\sigma_\varepsilon^2}{E[V(Y)|X]} \\
&= \frac{N\sigma_{\tilde{\beta}}^2}{E[V(Y)|X]}\sum_k\left(\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2-\frac{1}{N}\right)+\frac{M\sigma_{\tilde{\beta}}^2}{E[V(Y)|X]}+\frac{\sigma_\varepsilon^2}{E[V(Y)|X]} \\
&= N\frac{h_g^2}{M}\sum_k\left(\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2-\frac{1}{N}\right)+1
\end{aligned}$$

A.1.4 Removing the conditioning on X

When analyzing summary statistics, we do not have access to the true value of X, and so we need to compute the expectation of χ_j^2 treating X as random and integrating it out. To do this, we use the law of total expectation, and so the relevant quantity is $E\left[\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2\right]$. We would like our method to be applicable in the most general circumstances, and so we do not want to assume a particular distribution on X, or even that its rows are drawn i.i.d. from some distribution. Instead, we will let W_j denote the set of SNPs in an LD window around j, and we will make three assumptions that will allow us to complete our derivation:

1. There is a c such that for $k \notin W_j$, we have $E\left[\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2\right] \approx c$, and the approximation is good enough that $N\frac{h_g^2}{M}\sum_{k\notin W_j}\left(E\left[\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2\right]-c\right)$ is negligible. If there is no structure or relatedness in our samples (and if N is high enough that the difference between standardization in the population and in our sample is negligible), then c can be shown to be $1/N$.

2. For $k \in W_j$, there is a value R_{jk} satisfying $R_{jk} \approx E\left[\left(\frac{\tilde{X}_j^T \tilde{X}_k}{N}\right)^2\right] - c$, where the approximation is good enough that $N\frac{h_g^2}{M}\sum_{k\in W_j}\left(E\left[\left(\frac{\tilde{X}_j^T \tilde{X}_k}{N}\right)^2\right] - c - R_{jk}^2\right)$ is negligible. Note that if the rows of X are drawn i.i.d. from some distribution and R_{jk} is the correlation between SNPs j and k in this underlying distribution, and if $|W_j|$ is small compared to M, then this condition in satisfied.

We can now apply the law of total expectation to complete the derivation:

$$\begin{aligned}
E[\chi_j^2] &\approx N\frac{h_g^2}{M}\sum_k\left(E\left[\left(\frac{\tilde{X}_j^T \tilde{X}_k}{N}\right)^2\right] - \frac{1}{N}\right) + 1 \\
&= N\frac{h_g^2}{M}\sum_k\left(E\left[\left(\frac{\tilde{X}_j^T \tilde{X}_k}{N}\right)^2\right] - c\right) + N\frac{h_g^2}{M}\sum_k\left(c - \frac{1}{N}\right) + 1 \\
&\approx N\frac{h_g^2}{M}\sum_{k\in W_j}\left(E\left[\left(\frac{\tilde{X}_j^T \tilde{X}_k}{N}\right)^2\right] - c\right) + Nh_g^2\left(c - \frac{1}{N}\right) + 1 \\
&\approx N\frac{h_g^2}{M}\sum_{k\in W_j} R_{jk}^2 + Nh_g^2\left(c - \frac{1}{N}\right) + 1 \\
&= N\frac{h_g^2}{M}\sum_{k\in W_j} R_{jk}^2 + Na + 1,
\end{aligned}$$

where $a = h_g^2(c - 1/N)$. Letting

$$\ell_j = \sum_{k\in W_j} R_{jk}^2,$$

denote the LD score of SNP j, we obtain the main LD score regression equation:

$$E[\chi_j^2] \approx N\frac{h_g^2}{M}\ell_j + Na + 1. \tag{A4}$$

We typically estimate ℓ_j using a reference panel, and we estimate h_g^2 via weighted regression of χ_j^2 on $\ell(j)$, evaluating significance with block jackknife across SNPs.

A.2 LD score regression in the presence of covariates

We will now discuss LD score regression for a quantitative trait, in the presence of covariates. For a treatment of LD score regression for case-control traits with covariates**?**.

A.2.1 Summary statistics

Let C denote an $N \times K$ matrix of covariates, each column centered to mean zero. In a GWAS of a quantitative trait with covariates, we typically fit the model

$$Y = X_j\beta_{SNP,j}^{(marg)} + C\beta_{cov,j}^{(marg)} + \varepsilon_j^{(marg)}, \tag{A5}$$

where $\beta_{SNP,j}^{(marg)}$ is the marginal effect size of SNP j and $\beta_{cov,j}^{(marg)}$ is the effect size vector of the covariates.

The chi-square statistic is equal to

$$\chi_j^2 = \left(\hat{\beta}_{SNP,j}^{(marg)}\right)^2 / \hat{s}_j^2, \tag{A6}$$

where $\hat{\beta}_{SNP,j}^{(marg)}$ is the least-squares estimate of $\beta_{SNP,j}^{(marg)}$, and

$$\hat{s}_j^2 = \hat{\sigma}_{(marg)}^2 (A_j^T A_j)^{-1}{}_{11},$$

where A_j is the design matrix, given by $A_j = (X_j\, C)$, $(A_j^T A_j)^{-1}{}_{11}$ denotes the upper left entry of the matrix $(A_j^T A_j)^{-1}$, and $\hat{\sigma}_{(marg),j}^2$ is again an estimate of $\sigma_{(marg),j}^2$.

Let $P = I - C(C^T C)^{-1} C^T$. By the Frisch-Waugh-Lovell theorem, we have

$$\hat{\beta}_{SNP,j}^{(marg)} = \frac{(PX_j)^T PY}{(PX_j)^T PX_j},$$

and by block matrix inversion, we have

$$(A_j^T A_j)_{11}^{-1} = \frac{1}{(PX_j)^T (PX_j)}.$$

Again assuming that the effect size $\beta_{SNP,j}^{(marg)}$ is small, we have

$$\hat{\sigma}_{(marg)}^2 \approx \frac{1}{N} (PY)^T PY.$$

Let $V(PX_j) = ((PX_j)^T PX_j)/N$ and $V(PY) = (PY)^T PY/N$, and let $\tilde{X}_j = PX_j/\sqrt{V(PX_j)}$, and $\tilde{Y} = PY/\sqrt{V(PY)}$. Then, we can rewrite:

$$\chi_j^2 \approx \frac{1}{N} \left(\tilde{X}_j^T \tilde{Y}\right)^2 \tag{A7}$$

A.2.2 Deriving the expression for $E[\chi_j^2|X]$

In cov-LDSC, we assume that there are covariates in our GWAS model (Eq (A1)) and we include the same set of covariates in the polygenic model that we would like to fit:

$$Y = X\beta + C\beta_{cov} + \varepsilon, \tag{A8}$$

where Y, X, β, C, and ε are as before. Note that under this polygenic model,

$$PY = PX\beta + P\varepsilon.$$

Let $\tilde{\beta}_j = \beta_j \sqrt{V(X_j)}$. Note that $PX\beta = \tilde{X}\tilde{\beta}$. We will model $\tilde{\beta}_j$ as random with mean zero and variance $\sigma^2_{\tilde{\beta}}$. Now we have

$$\begin{aligned}
E[V(PY)|X] &= \frac{1}{N}E[(PY)^T PY|X] \\
&= \frac{1}{N}E\left[(PX\beta + P\varepsilon)^T (PX\beta + P\varepsilon)|X\right] \\
&= \frac{1}{N}E\left[\left(\tilde{X}\tilde{\beta} + P\varepsilon\right)^T \left(\tilde{X}\tilde{\beta} + P\varepsilon\right)|X\right] \\
&= \frac{1}{N}E[\tilde{\beta}^T\tilde{X}^T\tilde{X}\tilde{\beta}|X] + \frac{1}{N}E[(\varepsilon^T P^T P\varepsilon] \\
&= \frac{1}{N}\sum_{j,k} E\left[\tilde{\beta}_j (\tilde{X}^T\tilde{X})_{j,k}\tilde{\beta}_k|X\right] + \frac{1}{N}\sum_{j,k} E\left[\varepsilon_j \left(P^T P\right)_{j,k}\varepsilon_k\right] \\
&= \frac{1}{N}\sum_{j\neq k} E\left[\tilde{\beta}_j\right] E\left[\tilde{\beta}_k\right] (\tilde{X}^T\tilde{X})_{j,k} + \frac{1}{N}\sum_j E\left[\tilde{\beta}_j^2\right] (\tilde{X}^T\tilde{X})_{j,j} \\
&\qquad + \frac{1}{N}\sum_{j\neq k} E\left[\tilde{\varepsilon}_j\right] E\left[\tilde{\varepsilon}_k\right] (P^T P)_{j,k} + \frac{1}{N}\sum_j E\left[\varepsilon_j^2\right] (P^T P)_{j,j} \\
&= 0 + \frac{1}{N}\sum_j \sigma^2_{\tilde{\beta}} (\tilde{X}^T\tilde{X})_{j,j} + \sigma^2_\varepsilon + 0 + \frac{1}{N}\sum_j \sigma^2_\varepsilon (P^T P)_{j,j} \\
&= M\sigma^2_{\tilde{\beta}} + \sigma^2_\varepsilon \frac{N-K}{N}
\end{aligned}$$

where K is the rank of C. If K is small compared to N, as is typical of most GWAS, then we can say that

$$E[V(PY)|X] \approx M\sigma^2_{\tilde{\beta}} + \sigma^2_\varepsilon.$$

We will let h^2_g denote $M\sigma^2_{\tilde{\beta}}/E[V(PY)|X]$. It will again be convenient to have

$$\begin{aligned}
E\left[(\tilde{X}_j^T P\varepsilon)^2|X\right] &= E\left[\left(\frac{1}{\sqrt{V(PX_j)}} X_j^T P^T P\varepsilon\right)^2 |X\right] \\
&= E\left[\left(\frac{1}{\sqrt{V(PX_j)}} X_j^T P^T \varepsilon\right)^2 |X\right] \\
&= E\left[\left(\tilde{X}_j^T \varepsilon\right)^2 |X\right] \\
&= \tilde{X}_j^T E\left[\varepsilon\varepsilon^T\right] \tilde{X}_j \\
&= \sigma^2_\varepsilon \tilde{X}_j^T \tilde{X}_j \\
&= N\sigma^2_\varepsilon.
\end{aligned}$$

Now we have:

$$\begin{aligned}
E[\chi_j^2|X] &\approx \frac{1}{N}E\left[\left(\tilde{X}_j^T\tilde{Y}\right)^2|X\right] \\
&= E\left[\frac{1}{NV(PY)}\left(\tilde{X}_j^T PY\right)^2|X\right] \\
&\approx \frac{1}{NE\left[V(PY)|X\right]}E\left[\left(\tilde{X}_j^T(PX\beta+P\varepsilon)\right)^2|X\right] \\
&= \frac{1}{NE\left[V(PY)|X\right]}E\left[\left(\tilde{X}_j^T(\tilde{X}\tilde{\beta}+P\varepsilon)\right)^2|X\right] \\
&= \frac{1}{NE\left[V(PY)|X\right]}\sum_k(\tilde{X}_j^T\tilde{X}_k)^2E\left[\tilde{\beta}_k^2\right]+\frac{1}{NE\left[V(PY)|X\right]}E\left[(\tilde{X}_j^TP\varepsilon)^2|X\right] \\
&= \frac{N\sigma_{\tilde{\beta}}^2}{E\left[V(PY)|X\right]}\sum_k\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2+\frac{\sigma_\varepsilon^2}{E\left[V(PY)|X\right]} \\
&= \frac{N\sigma_{\tilde{\beta}}^2}{E[V(PY)|X]}\sum_k\left(\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2-\frac{1}{N}\right)+\frac{M\sigma_{\tilde{\beta}}^2}{E[V(PY)|X]}+\frac{\sigma_\varepsilon^2}{E\left[V(PY)|X\right]} \\
&\approx \frac{Nh_g^2}{M}\sum_k\left(\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2-\frac{1}{N}\right)+1
\end{aligned}$$

A.2.3 Removing the conditioning on X

We will make the same two assumptions as for LD score regression without covariates.

1. There is a c such that for $k \notin W_j$, we have $E\left(\frac{X_j^TX_k}{N}\right)^2 \approx c$. One way to formalize the notion that C captures all structure in X is that $c = 1/N$ in this case.

2. For $k \in W_j$, we have access, for example from a reference panel, to an estimate R_{jk} satisfying $R_{jk} \approx E\left(\frac{X_j^TX_k}{N}\right)^2 - c$. When X contains admixture or other structure, correlation as estimated from a reference panel may not suffice. In that case, we can set R_{jk} to be $\left(\frac{\tilde{X}_j^T\tilde{X}_k}{N}\right)^2$, or an estimate of that quantity from a random subsample of the GWAS. We note also that even if window size is 30 cM, this is still only approximately 1% of the genome, and so $|W_j|$ is still small compared to M.

With these assumptions satisfied, the rest of the derivation is identical to the case without covariates.

Appendix B. In-sample versus out-of-sample LD

To test the reliability of using an out-of-sample reference LD panel for cov-LDSC applications, we first examined the performance of out-of-sample LD scores obtained from 1,000 samples with a perfectly matching demographic history in the simulated genotypes. cov-LDSC yielded less biased estimates when using 1,000 samples in an out-of-sample reference panel with a perfectly matching population structure (**Figure S11**). Next, we tested the accuracy of heritability estimates and type I error of enrichment analysis when using 1000 Genomes Project Admixed American (AMR) samples to obtain out-of-sample LD scores. When using the AMR panel as a reference panel for the SIGMA cohort, we observed a less biased h_g^2 estimate ($P = 0.33$, **Figure 2(d)**), However, as we decreased the number of samples included in the subsampling, the cov-LDSC regression intercepts deviated further from one (**Figure S10(d)**). This is probably due to attenuation bias from noisily estimated LD scores at $N < 1,000$. We observed similar tissue type specific enrichment results for BMI, height and T2D (**Figure S20**). We further assessed the power and biases of using 1000 Genomes AMR samples as an external reference panel when applying it in the SIGMA cohort for tissue type specific analysis via simulation. We observed well calibrated type I error and similar power compared to in-sample LD reference panel (**Figure S19**). This suggested that the AMR panel included in the 1000 Genomes Project has similar demographic history compared to the SIGMA cohort (**Figure S6**, **S22**).

Next, we explored the feasibility of applying 1000 Genomes AMR samples in heritability estimation and its enrichment analyses in the 23andMe cohort. We obtained stratified LD scores using 1000 Genomes AMR samples ($N = 347$) and applied it on summary statistics obtained from 23andMe. In contrast to the SIGMA cohort, we discovered total heritability estimates are significantly different from those estimated using in-sample LD scores (**Table S12**) and discovered no significant tissue type enrichment (**Figure S21**). This suggested that 1000 Genome AMR samples might have different demographic history compared to 23andMe samples (**Figure S23**).

We therefore caution that when using 1000 Genomes or any out-of-sample reference panels for a specific admixed cohort, users should ensure that the demographic histories are shared between the reference and the study cohort. We highly recommend computing in-sample LD scores on a randomly chosen subset of at least 1,000 individuals from a GWAS. We also strongly encourage cohorts to release their summary statistics and in-sample covariate-adjusted LD scores at the same time to facilitate future studies.