# Polygenic Risk Score Analysis on Height GWAS data

Here we show an example of our LDpred2 pipeline for height PRS on an example public data-set from github user @choishingwan.

Analysis in this tutorial is performed by running our pipeline sequentially on a single computer (a desktop, laptop, or a single interactive node on a cluster). The pipeline iteself, implemented in the SoS workflow language, can be configured to run in parallel on a high performance computing cluster environment. Please read the SoS documentation on how to configure the software and workflow to efficiently perform the analysis for real-world data.

#### Data used

#### Reference panel

Obtained via download\_1000G() in bigsnpr.

We extracted unrelated European individuals (~500 samples) and ~1.7M SNPs in common with either HapMap3 or the UK Biobank. Classification of European population can be found at IGSR. European individuals ID are from IGSR data portal.

#### GWAS summary statistics data

Height.QC.gz, from public data-set provided by github user @choishingwan, of height GWAS in European samples.

#### Target test data

EUR.height, EUR.cov, and EUR.eigenvec contain phenotypes, covariates and genotype principle components of samples. EUR.QC.\* contain the corresponding genotypes, with ~400,000 variants. We have thinned the genotype data to ~150,000 variants for speeding up the examples used in this tutorial. The resulting genotypes are in EUR\_prune.\*.

#### PRS Models applied

Auto model runs the algorithm for 30 different p (the proportion of causal variants) values range from 10e-4 to 0.9, and heritability  $h^2$  from LD score regression as initial value.

Grid model tries a grid of parameters p, ranges from 0 to 1 and three  $h^2$  which are 0.7/1/1.4 times of initial  $h^2$  estimated by LD score regression.

### Data preparation

Please download the tutorial data and pipeline script to your computer. We have pre-downloaded

and extracted the European genotypes from 1000 Genomes, as well as genetic map data. We have preprocessed the GWAS data as follows, to fit in our pipeline.

Please do not run any code from this section. The data bundle above contains all the preprocessed files for you to start the PRS analysis (next section). Code below are documented here for book-keeping and reproducibility.

#### Summary statistics formatting

```
In [ ]:
          sumstats <- bigreadr::fread2("GWAS data/Height.QC.gz")</pre>
          # LDpred2 require the header to follow the exact naming
          names(sumstats) <-</pre>
              c("chr",
              "pos",
              "rsid",
              "a1",
              "a0",
              "n eff",
              "beta se",
              "p",
              "OR",
              "INFO",
              "MAF")
          # Transform the OR into log(OR)
          sumstats$beta <- log(sumstats$OR)</pre>
In [ ]:
          saveRDS(sumstats, "GWAS data/Height.QC.rds")
```

```
Savenus (Sams caes, Sans_act, No.1911. Qc.11as )
```

#### Phenotype and covariates formatting

```
In [ ]:
         options(stringsAsFactors=F)
         fam = read.table("GWAS data/EUR prune.fam", header=F)
         colnames(fam) = c("FID", "IID", "PID", "MID", "S", "D")
         pheno = read.table("GWAS data/EUR.height", header=T)
         covar = read.table("GWAS data/EUR.cov", header=T)
         pcs = read.table("GWAS data/EUR.eigenvec", header=F)
         colnames(pcs) = c("FID", "IID", "PC1", "PC2", "PC3", "PC4", "PC5", "PC6")
In [ ]:
         require(dplyr)
         pheno_out = left_join(fam, pheno, by = c("FID", "IID"))
         pheno out = left join(pheno out, covar, by = c("FID", "IID"))
         pheno_out = left_join(pheno_out, pcs, by = c("FID", "IID"))
In [ ]:
         write.table(pheno out[, "Height"], "GWAS data/EUR.height.matched.txt", col.names
         write.table(pheno out[, c("Sex",'PC1', 'PC2', 'PC3', 'PC4', 'PC5', 'PC6')], "GWA
```

The directory should have the following:

```
In [1]: tree
```

```
1000G.EUR
     1000G.EUR.bed
     1000G.EUR.bim
     1000G.EUR.fam
 GWAS data
   EUR.cov
    EUR.cov.matched.txt
     EUR.eigenvec
     EUR.height
     EUR.height.matched.txt
     EUR_prune.bed
     EUR_prune.bim
     EUR_prune.fam
    EUR_prune.log
     Height.QC.gz
    Height.QC.rds
 height results
     chr10.OMNI.interpolated_genetic_map
     chr11.0MNI.interpolated_genetic_map
    chr12.0MNI.interpolated_genetic_map
    - chr13.0MNI.interpolated_genetic_map
    - chr14.0MNI.interpolated_genetic_map

    chr15.0MNI.interpolated genetic map

    chr16.0MNI.interpolated genetic map

    chr17.0MNI.interpolated genetic map

    - chr18.0MNI.interpolated_genetic_map

    chr19.0MNI.interpolated genetic map

    chr1.0MNI.interpolated_genetic_map
    - chr20.0MNI.interpolated_genetic_map
    - chr21.0MNI.interpolated_genetic_map

    chr22.0MNI.interpolated genetic map

    chr2.0MNI.interpolated genetic map

    chr3.0MNI.interpolated_genetic_map
    chr4.0MNI.interpolated_genetic_map
    chr5.0MNI.interpolated_genetic_mapchr6.0MNI.interpolated_genetic_map
    - chr7.0MNI.interpolated_genetic_map
    chr8.0MNI.interpolated_genetic_map

    chr9.0MNI.interpolated genetic map

ldpred2 example.ipynb
- ldpred.ipynb
```

3 directories, 38 files

#### Analysis of height GWAS data

We set the work directory to height\_results folder (to be created by the workflow). This will also be used as part of the filenames in the outputs to identify this analysis.

```
In [1]: work_dir="height_results"
```

#### Step 1: QC on reference panel

Here we assume the GWAS genotype data EUR.\* has already been QC-ed. We perform here QC for reference panel,

```
sos run ldpred.ipynb snp_qc \
    --cwd $work_dir \
    --genoFiles 1000G.EUR/1000G.EUR.bed
```

```
INFO: Running basic QC filters: Filter SNPs and select individuals INFO: basic QC filters is completed.
INFO: basic QC filters output: /home/jovyan/work/height_results/1000G.EUR.height_results.bed
INFO: Workflow snp_qc (ID=015cfd3cc381496b) is executed successfully with 1 completed step.
```

The output of a command in our pipeline is highlighted in the green text above, in this case, /home/jovyan/work/height\_results/1000G.EUR.height\_results.bed . These are typically intermediate files generated and kept by the pipeline program. In this tutorial we will show contents from these outputs only when they are relevant to the final results, although in practice users are always encouraged to check these immediate files to ensure of no obvious sign of problems in each analysis step.

## Step 2: Intersect SNPs among summary stats, reference panel and target data

SNPs shared between summary statistics, reference panels and target genotype data (for which PRS will be computed) are extracted. Genetic distances will be computed for each variant using interpolated genetic map. **This step can take a bit of time to execute**.

```
In [3]:
         sos run ldpred.ipynb snp intersect \
              --cwd $work dir \
              --ss GWAS data/Height.QC.rds \
              --genoFiles $work_dir/1000G.EUR.$work_dir.bed GWAS_data/EUR_prune.bed
        INFO: Running snp intersect 1: SNP intersect of summary stats and genotype data
        INFO: snp_intersect_1 is completed.
INFO: snp_intersect_1 output: /ho
                                          /home/jovyan/work/height results/Height.QC.inter
        sect.rds /home/jovyan/work/height results/Height.QC.intersect.snplist
        INFO: Running snp_intersect_2:
        INFO: snp intersect 2 is completed (pending nested workflow).
        INFO: Running preprocess_1: Filter SNPs and select individuals
        INFO: preprocess_1 (index=1) is completed.
        INFO: preprocess_1 (index=0) is completed.
INFO: preprocess_1 output: /home/jovyan/
                                     /home/jovyan/work/height results/1000G.EUR.height r
        esults.snp intersect.extracted.bed /home/jovyan/work/height results/EUR prune.sn
        p intersect.extracted.bed in 2 groups
        INFO: Running convert PLINK to bigsnpr format with missing data mean imputed:
        INFO: convert PLINK to bigsnpr format with missing data mean imputed (index=0) i
        s completed.
        INFO: convert PLINK to bigsnpr format with missing data mean imputed (index=1) i
        s completed.
        INFO: convert PLINK to bigsnpr format with missing data mean imputed output:
        home/jovyan/work/height_results/1000G.EUR.height_results.snp_intersect.extracte
        d.bk /home/jovyan/work/height_results/1000G.EUR.height_results.snp_intersect.ext
         racted.rds... (4 items in 2 groups)
        INFO: snp intersect 2 output:
                                          /home/jovyan/work/height results/1000G.EUR.heigh
         t_results.snp_intersect.extracted.bed /home/jovyan/work/height_results/1000G.EU
        R.height_results.snp_intersect.extracted.rds... (4 items)
        INFO: Workflow snp intersect (ID=f5859b94a542bf56) is executed successfully with
        4 completed steps and 6 completed substeps.
```

```
In [4]:
```

```
cat $work_dir/Height.QC.intersect.stdout
```

#### Step 3: Harmonize alleles for shared SNPs

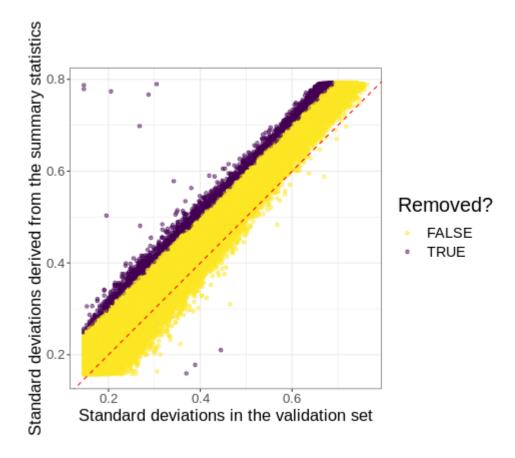
To handle major/minor allele, strand flips and consequently possible flips in sign for summary statistics.

```
In [5]:
         sos run ldpred.ipynb snp match \
             --cwd $work dir \
             --reference_geno $work_dir/1000G.EUR.$work_dir.snp_intersect.extracted.rds \
             --ss GWAS data/Height.QC.rds
        INFO: Running snp match:
        INFO: snp_match is completed.
                                   /home/jovyan/work/height results/Height.QC.snp matche
        INFO: snp match output:
        d.rds /home/jovyan/work/height results/Height.QC.snp matched.snplist
        INFO: Workflow snp match (ID=aa8f5f3cb5665126) is executed successfully with 1 c
        ompleted step.
       Step 4: Summary statistics quality control (optional)
        Please refer to documentation in the pipeline notebook ldpred.ipynb for an explanation of
       summary statistics QC.
In [6]:
         sos run ldpred.ipynb sumstats_qc \
             --cwd $work dir \
             --reference geno $work dir/1000G.EUR.$work dir.snp intersect.extracted.rds \
             --ss $work dir/Height.QC.snp matched.rds \
             --sdv 1
        INFO: Running sumstats qc:
        INFO: sumstats_qc is completed.
        INFO: sumstats qc output:
                                     /home/jovyan/work/height results/Height.QC.snp match
        ed.qc.png /home/jovyan/work/height results/Height.QC.snp matched.qc.snplist...
         (3 items)
        INFO: Workflow sumstats qc (ID=dbbf3e7458e2f8a0) is executed successfully with 1
        completed step.
```

> height\_results/Height.QC.snp\_matched.gc.png (63.9 KiB):

%preview height\_results/Height.QC.snp\_matched.qc.png

In [7]:



From the results, we observe only a few outliers significantly deviating from the majority of the variants. We suspect that quality control recommanded in LDpred2 manuscript may be too stringent and should not be needed for this data-set. Notice that as the manuscript pointed out, the quality control procedure is not suitable for meta-analyzed GWAS summary statistics.

#### Step 5: Calculate LD matrix and fit LDSC model

As an illustration, hereafter all analysis are performed on both summary statistics before QC in Step 4:

```
In [9]:
sos run ldpred.ipynb ldsc \
    --cwd $work_dir \
```

```
--ss $work dir/Height.QC.snp matched.qc.rds \
              --reference-geno $work dir/1000G.EUR.$work dir.snp intersect.extracted.rds
         INFO: Running ldsc:
         INFO: ldsc is completed.
                               /home/jovyan/work/height results/Height.QC.snp matched.qc.l
         INFO: ldsc output:
         d.rds
         INFO: Workflow ldsc (ID=bacc9e382c830fa4) is executed successfully with 1 comple
         ted step.
        Step 6: Estimate posterior effect sizes and PRS
         For QC-ed data, we perform 3 PRS models implemented in 1dpred2. We demonstrate the
        infinitesimal model here:
In [10]:
          sos run ldpred.ipynb inf prs \
              --cwd $work dir \
              --ss $work dir/Height.QC.snp matched.qc.rds \
              --target-geno $work_dir/EUR_prune.snp_intersect.extracted.rds \
              --ldsc $work dir/Height.QC.snp matched.qc.ld.rds
         INFO: Running inf prs:
         INFO: inf_prs is completed (pending nested workflow).
         INFO: Running prs_core:
         INFO: prs_core is completed.
         INFO: prs core output:
                                  /home/jovyan/work/height results/Height.QC.snp matched.
         qc.inf_prs.rds
         INFO: inf_prs output: /home/jovyan/work/height_results/Height.QC.snp_matched.q
         c.inf_prs.rds
         INFO: Workflow inf_prs (ID=1219f13000c52cc3) is executed successfully with 2 com
         pleted steps.
         Here we examine the PRS generated from this command,
```

cat \$work dir/Height.QC.snp matched.qc.inf prs.stdout

dat = readRDS('height results/Height.QC.snp matched.qc.inf prs.rds')

hist(dat\$prs, breaks=20, main="PRS distribution", xlab="PRS")

[1] "104564 SNPs are used for PRS calculations"

In [11]:

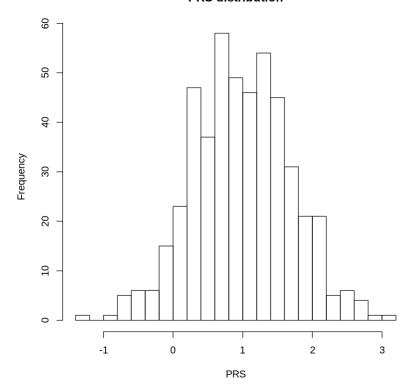
In [12]:

In [23]:

names(dat)

'beta'
 'prs'

#### PRS distribution



Commands below will execute the "auto" model and "grid" model implemented in LDpred2 . They are typically more powerful but also computationally intensive. We document them here without executing:

We then run the infinitesimal model for original data:

INFO: Running Int\_prs.
INFO: inf\_prs is completed (pending nested workflow).
INFO: Running prs\_core:

```
inf_prs.rds
INFO: inf_prs output: /home/jovyan/work/height_results/Height.QC.snp_matched.i
nf_prs.rds
INFO: Workflow inf_prs (ID=f266994e074ae431) is executed successfully with 2 com
pleted steps.

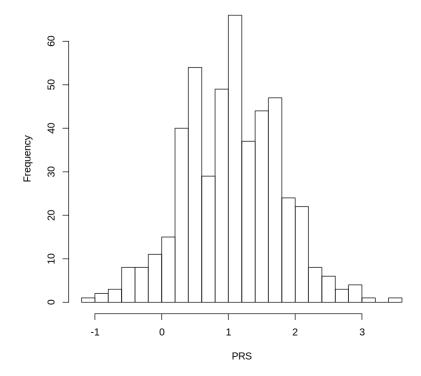
In [25]: cat $work_dir/Height.QC.snp_matched.inf_prs.stdout
[1] "109963 SNPs are used for PRS calculations"

In [26]: dat = readRDS('height_results/Height.QC.snp_matched.inf_prs.rds')
hist(dat$prs, breaks=20, main="PRS distribution (without summary statistics QC)'
```

/home/jovyan/work/height results/Height.QC.snp matched.

#### PRS distribution (without summary statistics QC)

INFO: prs\_core is completed.
INFO: prs core output: /ho



Again, here are commands for "auto" and "grid" models:

```
--covFile GWAS_data/EUR.cov.matched.txt \
--response continuous
```

For grid model, in practice we should use another subset of individuals to train the model, independent from the subset to make PRS predictions. Here we use the same target genotype data only to illustrate the workflow.

#### Step 7: predict phenotypes

Baseline model: Trait ~ Sex + PCs

```
In [27]:
          sos run ldpred.ipynb pred eval \
              --cwd $work dir \
              --phenoFile GWAS data/EUR.height.matched.txt \
              --covFile GWAS data/EUR.cov.matched.txt \
              -- response continuous
         INFO: Running pred eval:
         INFO: pred eval is completed.
         INFO: pred eval output: /home/jovyan/work/height results/EUR.height.matched.ba
         seline.rds
         INFO: Workflow pred eval (ID=bd7c33f977855a7a) is executed successfully with 1 c
         ompleted step.
In [28]:
          res = readRDS("height_results/EUR.height.matched.baseline.rds")
          summary(res$fitted)
          res$summary
         Call:
         lm(formula = ., data = dat[train.ind, ])
         Residuals:
                        10
              Min
                              Median
                                           30
                                                   Max
         -1.88058 -0.57445 -0.02782 0.51809 2.60256
         Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
                                                      <2e-16 ***
         (Intercept) 168.78869 0.13623 1238.982
                                                      <2e-16 ***
         Sex
                       0.89443
                                   0.08528 10.488
         PC1
                      -0.99439
                                   0.90011
                                             -1.105
                                                      0.2700
                      -2.36803
         PC2
                                   0.94434
                                             -2.508
                                                      0.0126 *
         PC3
                       -0.67896
                                   0.95222
                                             -0.713
                                                      0.4763
         PC4
                       0.11199
                                   1.72585
                                              0.065
                                                      0.9483
         PC5
                       0.44029
                                   0.95044
                                              0.463
                                                      0.6435
         PC6
                        0.22226
                                   0.91901
                                              0.242
                                                      0.8090
         Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
         Residual standard error: 0.82 on 367 degrees of freedom
           (11 observations deleted due to missingness)
         Multiple R-squared: 0.244,
                                          Adjusted R-squared: 0.2296
         F-statistic: 16.92 on 7 and 367 DF, p-value: < 2.2e-16
              A tibble: 1 \times 3
         model
                   R2
                         MSE
          <chr>
                 <dbl>
                        <dbl>
          model 0.22956 0.81868
```

model

R2

**MSE** 

```
In [29]:
          sos run ldpred.ipynb pred eval \
              --cwd $work dir \
              --prs $work_dir/Height.QC.snp_matched.qc.inf_prs.rds \
              --phenoFile GWAS_data/EUR.height.matched.txt \
              --covFile GWAS data/EUR.cov.matched.txt \
              -- response continuous
          sos run ldpred.ipynb pred_eval \
              --cwd $work dir \
              --prs $work dir/Height.QC.snp matched.inf prs.rds \
              --phenoFile GWAS data/EUR.height.matched.txt \
              --covFile GWAS data/EUR.cov.matched.txt \
              -- response continuous
         INFO: Running pred eval:
         INFO: pred eval is completed.
         INFO: pred eval output:
                                  /home/jovyan/work/height results/EUR.height.matched.He
         ight.QC.snp matched.qc.inf prs.rds
         INFO: Workflow pred eval (ID=d4028a7c469c4955) is executed successfully with 1 c
         ompleted step.
         INFO: Running pred_eval:
         INFO: pred eval is completed.
         INFO: pred eval output:
                                    /home/jovyan/work/height results/EUR.height.matched.He
         ight.QC.snp matched.inf prs.rds
         INFO: Workflow pred eval (ID=4a11957219f352cc) is executed successfully with 1 c
         ompleted step.
In [30]:
          res = readRDS("height results/EUR.height.matched.Height.OC.snp matched.gc.inf pr
          summary(res$fitted)
          res$summary
         lm(formula = ., data = dat[train.ind, ])
         Residuals:
              Min
                         10
                              Median
                                                   Max
         -1.73015 -0.52593 -0.02821 0.47019 2.40833
         Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
                                   0.13481 1248.673
                                                      <2e-16 ***
         (Intercept) 168.33930
                                             11.050
                                                      <2e-16 ***
         Sex
                       0.86128
                                   0.07794
         PC1
                      -1.02292
                                   0.82162
                                             -1.245
                                                       0.214
         PC2
                      -1.32026
                                   0.87049
                                             -1.517
                                                       0.130
         PC3
                      -1.23730
                                   0.87159
                                             -1.420
                                                       0.157
         PC4
                      -0.33065
                                             -0.210
                                                       0.834
                                   1.57618
         PC5
                                              0.478
                       0.41502
                                   0.86756
                                                       0.633
         PC6
                       0.06739
                                   0.83906
                                              0.080
                                                       0.936
                                                      <2e-16 ***
         PRS
                       0.48896
                                   0.05666
                                              8.630
         Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
         Residual standard error: 0.7485 on 366 degrees of freedom
           (11 observations deleted due to missingness)
         Multiple R-squared: 0.3718,
                                          Adjusted R-squared: 0.3581
         F-statistic: 27.08 on 8 and 366 DF, p-value: < 2.2e-16
                A tibble: 1 \times 3
```

```
mddel
                      <db > < db > E
               <chr>
                      <dbl>
                            <dbl>
          model.inf_prs 0.35808 0.6695
In [31]:
          res = readRDS("height results/EUR.height.matched.Height.QC.snp matched.inf prs.
          summary(res$fitted)
          res$summary
          Call:
          lm(formula = ., data = dat[train.ind, ])
         Residuals:
               Min
                         10
                               Median
                                                     Max
          -1.76983 -0.49570 -0.03415 0.48238 2.46186
          Coefficients:
                       Estimate Std. Error t value Pr(>|t|)
                                                        <2e-16 ***
          (Intercept) 168.31863
                                    0.13505 1246.328
                                                        <2e-16 ***
          Sex
                        0.85878
                                    0.07772
                                              11.049
          PC1
                       -1.14006
                                    0.81936
                                               -1.391
                                                        0.1649
          PC2
                       -1.43282
                                    0.86602
                                              -1.654
                                                        0.0989
          PC3
                       -1.25347
                                    0.86909
                                              -1.442
                                                        0.1501
          PC4
                       -0.30386
                                    1.57142
                                              -0.193
                                                        0.8468
          PC5
                        0.44159
                                    0.86500
                                                0.511
                                                        0.6100
          PC6
                        0.13108
                                    0.83646
                                                0.157
                                                        0.8756
          PRS
                        0.47990
                                    0.05466
                                                8.780
                                                        <2e-16 ***
          Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
          Residual standard error: 0.7463 on 366 degrees of freedom
            (11 observations deleted due to missingness)
          Multiple R-squared: 0.3755,
                                           Adjusted R-squared: 0.3619
          F-statistic: 27.51 on 8 and 366 DF, p-value: < 2.2e-16
                 A tibble: 1 \times 3
               model
                         R2
                               MSE
               <chr>
                      <dbl>
                             <dbl>
          model.inf_prs 0.36185 0.69409
         Here are commands for running prediction with "auto" and "grid" models:
             sos run ldpred.ipynb pred eval \
                 --cwd $work dir \
                 --prs $work dir/Height.QC.snp matched.qc.auto prs.rds \
                 --phenoFile GWAS data/EUR.height.matched.txt \
                 --covFile GWAS data/EUR.cov.matched.txt \
                 --response continuous
             sos run ldpred.ipynb pred eval \
                 --cwd $work dir \
                 --prs $work dir/Height.QC.snp matched.auto prs.rds \
                 --phenoFile GWAS data/EUR.height.matched.txt \
                 --covFile GWAS data/EUR.cov.matched.txt \
```

--response continuous

```
sos run ldpred.ipynb pred_eval \
    --cwd $work_dir \
    --prs $work_dir/Height.QC.snp_matched.qc.grid_prs.rds \
    --phenoFile GWAS_data/EUR.height.matched.txt \
    --covFile GWAS_data/EUR.cov.matched.txt \
    --response continuous

sos run ldpred.ipynb pred_eval \
    --cwd $work_dir \
    --prs $work_dir/Height.QC.snp_matched.grid_prs.rds \
    --phenoFile GWAS_data/EUR.height.matched.txt \
    --covFile GWAS_data/EUR.cov.matched.txt \
    --response continuous
```

## Results summary

Following table shows adjusted R squared of height prediction models. "QC" refers to quality control in Step 4.

Compared to baseline model, higher R squared with PRS included implies PRS explains part of the variation of height in the target data-set. Higher R squared is observed without performing quality control for the summary statistics, as we already expected and discussed after seeing the results in Step 4.

QC?	# of SNPs	Baseline R2	Inf R2	Grid R2	Auto R2
Yes	104,564	0.2296	0.3581	-	-
No	109,963	0.2296	0.3619	-	-

#### Homework

Please complete the runs with grid and auto model and complete the table above.

Computation with these models are intensive and long-running; it is suggested that the analysis be performed in a powerful computing environment.