Lecture 2: Introduction to the PLINK Software for GWAS & Population Structure Inference

Instructors: Joelle Mbatchou and Loic Yengo

Summer Institute in Statistical Genetics 2022

PLINK Overview

- ▶ PLINK is a free, open-source whole genome association analysis toolset, designed to perform a range of basic, large-scale analyses in a computationally efficient manner:

  https://www.cog-genomics.org/plink/1.9/
  https://www.cog-genomics.org/plink/2.0/

- ▶ PLINK has numerous useful features for managing and analyzing genetic data

PLINK Overview

▶ Data management
  ▶ Read data in a variety of formats (BED, PGEN, BGEN, VCF,...)
  ▶ Convert between different formats
  ▶ Recode and reorder files
  ▶ Merge multiple genetic files
  ▶ Extracts subsets (SNPs or individuals)

PLINK Overview

► Summary statistics for quality control
  ► Allele & genotypes counts/frequencies
  ► Missing genotype rates
  ► Mendel error rate
  ► HWE tests
  ► Sample variant counts
  ► Inbreeding, IBS and IBD statistics for individuals and pairs of individuals

PLINK Overview

- ▶ Basic association testing
  - ▶ Standard allelic test & Fisher's exact test for case-control data
  - ▶ Linear and logistic regression
  - ▶ Dominant/recessive and general models
  - ▶ Family-based association tests (e.g. TDT)
  - ▶ Association conditional on one or more SNPs

PLINK Overview

- ▶ Gene-based tests of association
- ▶ Screen for epistasis
- ▶ Gene-environment interaction with continuous and dichotomous environments
- ▶ Meta-analysis
  - ▶ Automatically combine several generically-formatted summary files, for millions of SNPs

Input Files

- ▶ PLINK BED: Genotype data is a compressed binary file $(0/1)$
  - ▶ Fam File (.fam) contains sample information
  - ▶ Bim file (.bim) contains variant information
  - ▶ Bed file (.bed) contains the genotype data

Data Management

- ▶ Inclusion/Exclusion criteria options
  - ▶ `--keep mylist.txt, --remove mylist.txt`
  - ▶ `--extract mysnps.txt, --exclude mysnps.txt`
  - ▶ `--chr 2,6 --from rs273744 --to rs89883`
- ▶ Other data management options
  - ▶ `--make-bed, --export, --pmerge`
- ▶ Using files with phenotypes/covariates
  - ▶ `--pheno, --covar`

Quality Control (QC)

- ▶ Summary statistics options:
    - ▶ minor allele frequency (MAF): `--freq`
    - ▶ genotype counts: `--geno-counts`
    - ▶ SNP & individual missing rate: `--missing`
    - ▶ Hardy-Weinberg: `--hardy`
- ▶ Inclusion/Exclusion filters
    - ▶ MAF: `--maf`
    - ▶ MAC: `--mac`
    - ▶ SNP missing rate: `--geno`
    - ▶ Individual missing rate: `--mind`
    - ▶ Hardy-Weinberg: `--hwe`

Association Analysis with PLINK

With PLINK

▶ Association testing: `--assoc`, `--linear`, `--logistic`

▶ Conditional analysis: `--condition-list`

▶ GxE interaction: `--gxe`

With PLINK2

▶ Association testing: `--glm`

▶ Conditional analysis: `--condition-list`

▶ GxE interaction: `--glm interaction`

GWAS of Transferrin

- PLINK input files:
  - `Transferrin.bed`
  - `Transferrin.fam`
  - `Transferrin.bim`
- Rmd script file for Transferrin: available on course website
  - `assoc_ex03.Rmd`
- HELP: check the PLINK website documentation (very useful!)

  https://www.cog-genomics.org/plink/

Background: Population Structure

- ▶ **PLINK can also be used to infer population structure**
- ▶ Humans originally spread across the world many thousand years ago.
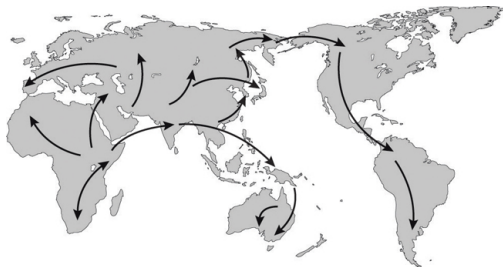- ▶ Migration and genetic drift led to genetic diversity between isolated groups.

Figure: https://science.education.nih.gov

Population Structure Inference

- ▶ Inference on genetic ancestry differences among individuals from different populations, or **population structure**, has been motivated by a variety of applications:
  - ▶ population genetics
  - ▶ genetic association studies
  - ▶ personalized medicine
  - ▶ forensics
- ▶ Advancements in array-based genotyping technologies have largely facilitated the investigation of genetic diversity at remarkably high levels of detail
- ▶ A variety of methods have been proposed for the identification of genetic ancestry differences among individuals in a sample using high-density genome-screen data.

Inferring Population Structure with PCA

▶ Principal Components Analysis (PCA) is the most widely used
   approach for identifying and adjusting for ancestry difference
   among sample individuals

▶ PCA applied to genotype data can be used to calculate
   principal components (PCs) that explain differences among
   the sample individuals in the genetic data

▶ The top PCs are viewed as continuous axes of variation that
   reflect genetic variation due to ancestry in the sample.

▶ Individuals with "similar" values for a particular top principal
   component will have similar ancestry for that axes.

Standard Principal Components Analysis (sPCA)

- ▶ sPCA is an unsupervised learning tool for dimension reduction in multivariate analysis.
- ▶ Widely used in genetics community to infer population structure from genetic data.
  - ▶ Belief that top principal components (PCs) will reflect population structure in the sample.
- ▶ Orthogonal linear transformation to a new coordinate system
  - ▶ sequentially identifies linear combinations of genetic markers that explain the greatest proportion of variability in the data
  - ▶ these define the axes (PCs) of the new coordinate system
  - ▶ each individual has a value along each PC
- ▶ EIGENSOFT (Price et al. 2006) is a popular implementation of PCA.

Data Structure

- ▶ Sample of n individuals, indexed by $i = 1, 2, \ldots, n$.
- ▶ Genome screen data on m genetic autosomal markers, indexed by $l = 1, 2, \ldots, m$.
- ▶ At each marker, for each individual, we have a genotype value, G_{il}.
  - ▶ Here we consider SNP data, so G_{il} takes values 0, 1, or 2, corresponding to the number of minor alleles.
- ▶ We center and standardize these genotype values:

$$z_{il} \;=\; \frac{G_{il} - 2\hat{p}_l}{\sqrt{2\hat{p}_l(1 - \hat{p}_l)}}$$

where $\hat{p}_l$ is an estimate of the minor allele frequency for marker l.

Genetic Correlation Estimation

▶ Create an $n \times m$ matrix, $\mathbf{Z}$, of centered and standardized genotype values, and from this, a $n \times n$ genetic correlation matrix (GRM):
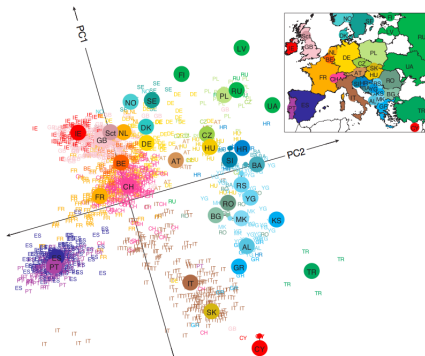
$$\widehat{\boldsymbol{\Psi}} = \frac{1}{m}\mathbf{Z}\mathbf{Z}^T$$

▶ $\widehat{\boldsymbol{\Psi}}_{ij}$ is an estimate of the genome wide average genetic correlation between individuals i and j.

▶ PCA is performed by obtaining the eigendecomposition $\widehat{\boldsymbol{\Psi}}$

Standard Principal Components Analysis (sPCA)

- ▶ Identify orthogonal axes of variation, i.e. linear combinations of SNPs, that best explain the genotypic variability between the n sample individuals.
- ▶ The result is:
  - ▶ a set of n length n eigenvectors, $(\mathbf{V}_1, \mathbf{V}_2, \ldots \mathbf{V}_n)$, where $\mathbf{V}_d$ is a column vector of coordinates of each individual along axis d
  - ▶ each principal component is a different linear combination of the m markers
  - ▶ and a corresponding set of n eigenvalues, $(\lambda_1 > \lambda_2 > \ldots > \lambda_n)$, in decreasing order.
  - ▶ The dth principal component (eigenvector) corresponds to eigenvalue λ_d, where λ_d is proportional to the percentage of variability in the genome-screen data that is explained by $\mathbf{V}_d$.
- ▶ These eigenvectors (PCs) are used as surrogates for population structure

PCA of Europeans

- Application of PCA in European samples (Novembre et al., *Nature* 2008)

- Among Europeans for whom all four grandparents originated in the same country, the first two PCs computed using 200k SNPs could map their country of origin quite accurately

Relatedness Confounds sPCA

▶ Recall that the GRM used by sPCA, $\widehat{\boldsymbol{\Psi}}_{ij}$, and is an estimate of the genome wide average genetic correlation between individuals i and j.

▶ It can be shown:

$$\boldsymbol{\Psi}_{ij} = 2 \left[\phi_{ij} + (1 - \phi_{ij}) A_{ij} \right]$$

  ▶ ϕ_{ij}: kinship coefficient - a measure of familial relatedness
  ▶ A_{ij}: a measure of ancestral similarity

▶ PCA is an unsupervised method; in related samples we don't know the correlation structure each eigenvector is reflecting
  ▶ If the only genetic correlation structure among individuals is due to ancestry, $\boldsymbol{\Psi}$ and the top PCs will capture this.
  ▶ If there is relatedness in the sample, the top PCs may reflect this or some combination of ancestry and relatedness.

▶ Association studies have known or cryptic relatedness!

sPCA: Best practices

- ▶ Apply QC to variants & samples:
  - ▶ Restrict to common variants (e.g. MAF ≥ 0.01)
  - ▶ Remove variants with high missing genotypes rates (e.g. ≥ 0.01)
  - ▶ Remove variants which fail HWE test (e.g. p-value $\leq 10^{-10}$)
  - ▶ Remove samples with high missing genotypes rates (e.g. ≥ 0.1)
  - ▶ Keep only variants on autosomal chromosomes
- ▶ Remove related individuals (e.g. 3rd degree related or closer)
- ▶ Prune variants in linkage disequilibrium (LD) (e.g. $r^2 \geq 0.2$)

R package bigsnpr

- ▶ Apply QC to variants & samples (relies on PLINK2)

  ```
  snp_plinkQC(plink.path, prefix.in, file.type="--bfile",
   maf = 0.01, geno = 0.1, mind = 0.1, hwe = 1e-10,
   autosome.only = TRUE )
  ```

- ▶ Remove related individuals (e.g. 3rd degree related or closer)
  ```
  extra.options = "--king-cutoff 0.0442"
  ```

- ▶ Compute PCs
    - ▶ Prune variants in linkage disequilibrium (LD) (e.g. $r^2 \geq 0.2$)
    - ▶ Removes long-range LD regions

  ```
  bed_autoSVD(obj.bed, thr.r2 = 0.2, k = 20)
  predict(object.svd)
  ```

- ▶ Project to remaining samples
  ```
  bed_projectSelfPCA(object.svd, obj.bed, ind.row)
  ```

R package bigsnpr

```
plot(obj.svd2, type = "scores", scores = 1:20, coeff = 0.4)
```
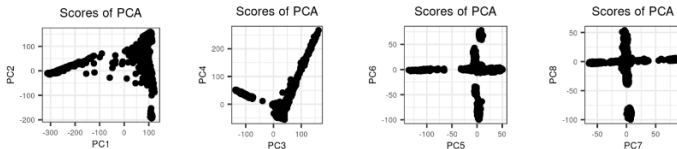


```
plot(obj.svd2, type = "loadings", loadings = 1:20, coeff = 0.4)
```
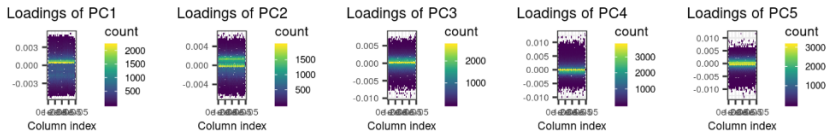

Figure: https://privefl.github.io/bigsnpr/articles/bedpca.html

References

▶ Patterson,N., Price, A.L., Reich, D. (2006) Population structure and eigenanalysis. *PLoS Genet.* **2**, e190.

▶ Novembre, J., Johnson, T., Bryc, K., Kutalik, Z., Boyko, A.R., Auton, A., Indap, A., King, K.S., Bergmann, S., Nelson, M.R. (2008). Genes mirror geography within Europe. *Nature* **456**, 98-101.

▶ Alexander, D.H., Novembre, J., Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**,1655-1664.

References

▶ Conomos MP, Miller M, Thornton T (2015). Robust Inference of Population Structure for Ancestry Prediction and Correction of Stratification in the Presence of Relatedness. *Genetic Epidemiology* **39**, 276-93

▶ Privé, F., Luu, K., Blum, M. G., McGrath, J. J., Vilhjálmsson, B. J. (2020). Efficient toolkit implementing best practices for principal component analysis of population genetic data. *Bioinformatics*, 36(16), 4449-4457.