GWAS Analysis for Behavioral Traits in Domestic Sheep

Training Material

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AUTH

07.05.2025

Introduction to GWAS
(Genome-Wide Association Studies)

What is GWAS?

A statistical approach based on genome variation testing associations between multiple genetic variants (across many genomes) and phenotypes of interest

Detection of differential allele frequencies of variants to reveal complex trait variation (Uffelmann, 2021)
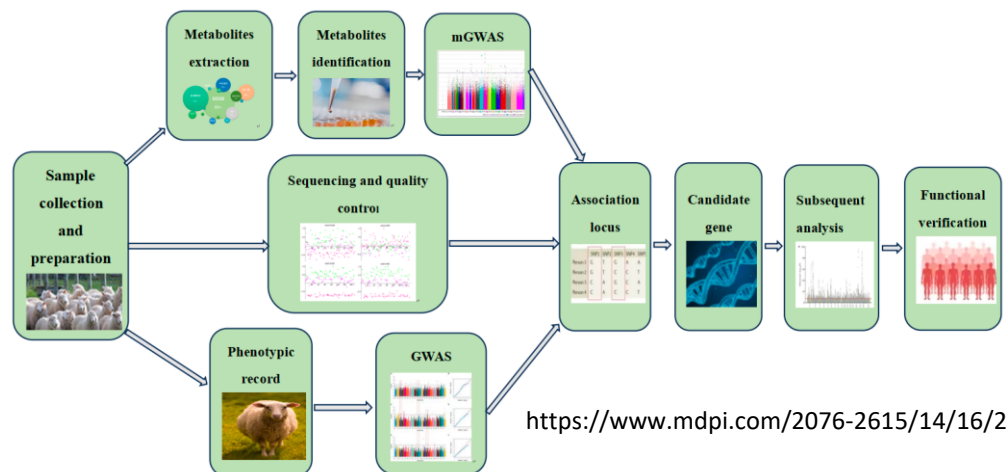
These variants are called causal (non-randomly linked) and partially control phenotypic variation

Individual 1
Maternal . . . CGATATTCC**T**ATCGAATGTC. . .
Paternal . . . CGATATTCC**C**ATCGAATGTC. . .

Individual 2
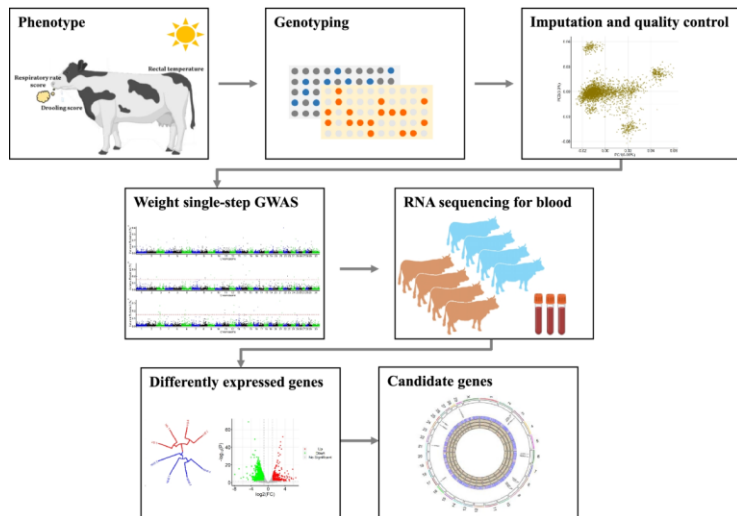Maternal . . . CGATATTCC**C**ATCGAATGTC. . .
Paternal . . . CGATATTCC**C**ATCGAATGTC. . .

Individual 3
Maternal . . . CGATATTCC**T**ATCGAATGTC. . .
Paternal . . . CGATATTCC**T**ATCGAATGTC. . .

Individual 4
Maternal . . . CGATATTCC**C**ATCGAATGTC. . .
Paternal . . . CGATATTCC**T**ATCGAATGTC. . .

Metabolites extraction → Metabolites identification → mGWAS

Sample collection and preparation → Sequencing and quality control → Association locus → Candidate gene → Subsequent analysis → Functional verification

Phenotypic record → GWAS

https://www.mdpi.com/2076-2615/14/16/2382

Applications in Livestock Genomics

Traits of economic interest (production, reproduction, health, longevity)

↓

Quantitative characters - continuous variation within/between populations

↓

Influenced by multiple genes with relative effects - polygenic inheritance

Increasing interest on adaptive and behavioral traits

↓

Understanding of local adaptation, animal resilience/behavior to environmental changes

**Implementation of genomic selection for genetic improvement**

WHAT IS PHENOTYPE

Phenotype (P) = Genotype (G) + Environment (E)

- **Phenotype** : Trait, observable characteristic of an animal

- **Genotype** : Genetic profile of an individual. We can act on it. Inherited

- **Environment** : Events around the individual. We can partially act on it. Not inherited

WHAT IS GENOTYPE

- **Genotype:** Determined by alleles/DNA segments across genome of an individual coding for functions related to the trait (also used to describe the alleles on a particular locus)

- **Haplotype:** A group of tightly linked alleles/DNA segments from a single parent inherited together

- **Haplotype Blocks:** haplotypes of different length are sometimes co-inherited (Linkage Disequilibrium)
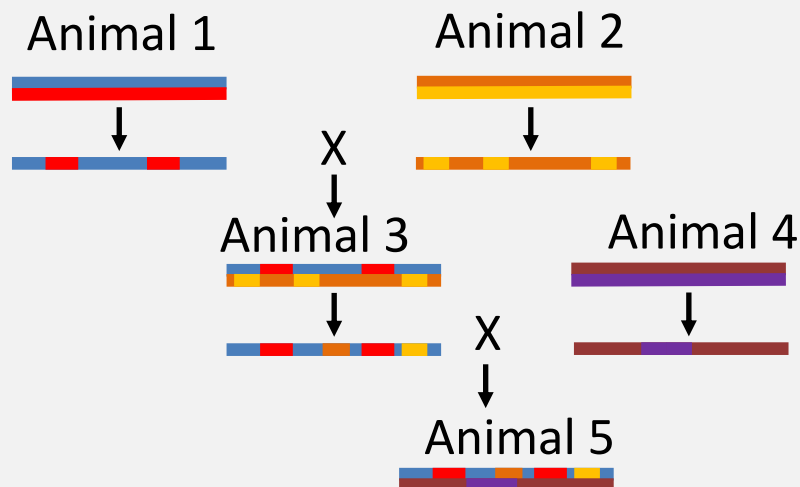
- **Recombination reduces LD (shorter haplotypes)**

- **Selection increase LD (longer haplotypes)**

WHAT IS GENETIC MARKER

- **Markers: Variable sites/sequences with known position on the genome**
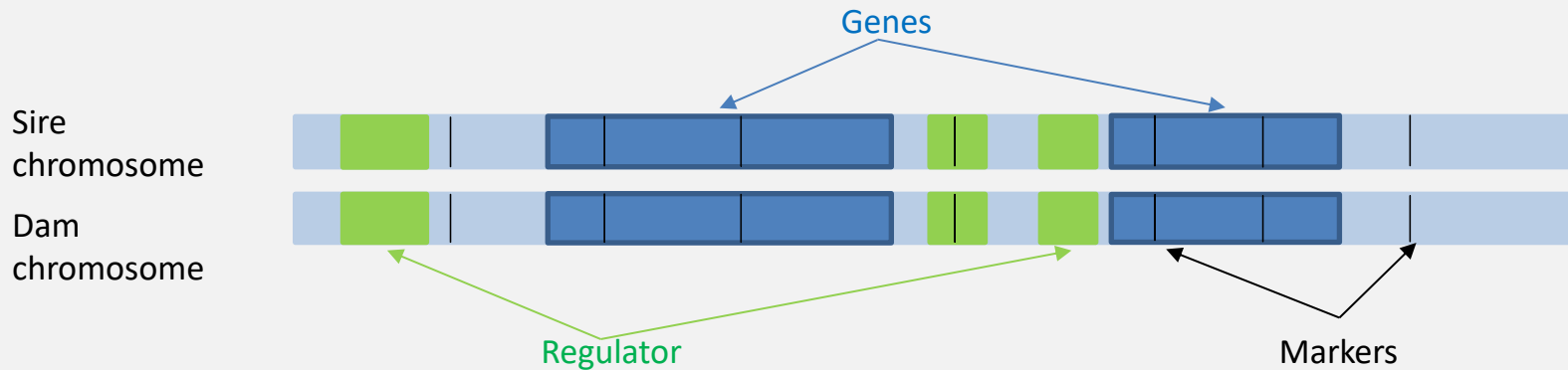
- **They can be linked with genes related to a trait of interest**
- **If they are linked with relevant genes, we can use them for selection purposes**
- **We can also use them to estimate the relationships between individuals**

High-throughput genotyping and GWAS
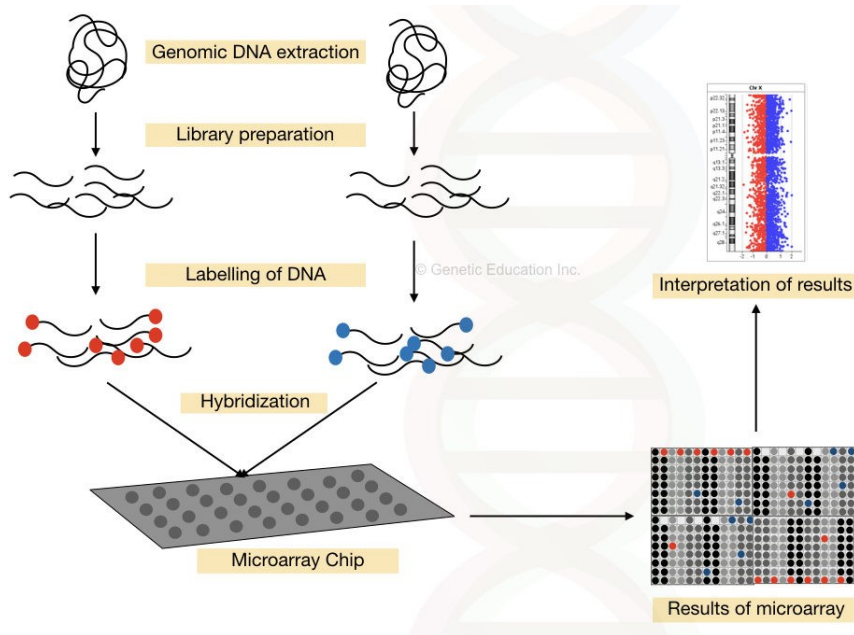
SNP arrays: Genotyping of Single Nucleotide Polymorphisms (SNPs) across the genome

Development of species-specific arrays of thousands or million markers

In livestock, multiple global breeds are used for array designation and validation

GWAS studies testing association between phenotypes and the total number of available SNPs

Classic Animal Model

Statistical methods first developed by Fisher (1918) and Wright (1921) to partition the observed phenotypic variation into variance components (genetic, environmental)

Phenotype (P) = Genotype (G) + Environment (E)

$$Y_i = X + u_i + \varepsilon$$

- **Phenotype (Y)** : Trait, observable characteristic of an individual

- **Fixed effect (X)** : Part of the environment clearly identified, eg: Farm, sex, year, …

- **Random effect (u)** : Animal genetic profile – estimated with pedigree information

- **Error (ε):** Residual due to imperfection of the model

GWAS Model

Test the association of each SNP marker with the phenotype

GWAS model ➔ **Y** = **X** + **_Marker effect_** + **_u_** + **_ε_**

- **Phenotype (Y)** : Trait, observable characteristic of an animal

- **Fixed effect (X)** : Environmental factors clearly identified, eg: Farm, sex, year, …

- **Marker (SNP) effect**: assessed for each SNP

- **Random effect (u)** : random (additive genetic) polygenic effect ⟶ Genomic Relatedness Matrix (GRM)

- **Error (ε):** Residual due to imperfection of the model

Control for genetic relatedness between individuals that may lead to false positive associations

GWAS Model

Manhattan plot

SNPs with different allele frequencies among subgroups of the study population

https://link.springer.com/article/10.1186/s12711-021-00682-7
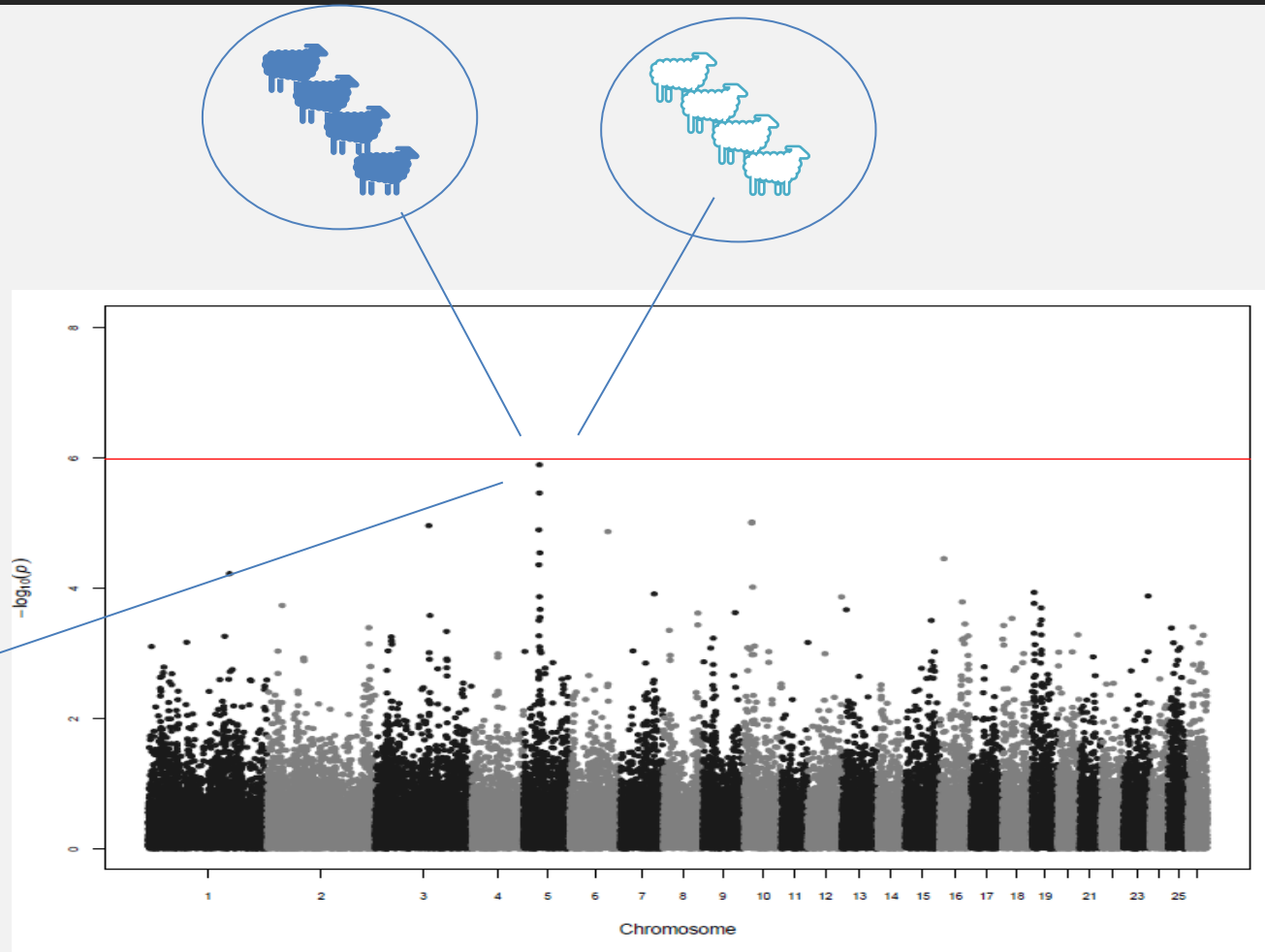
Case Study

Grazing behavior of mountainous Greek Boutsko sheep

Study Design – Overview

- Phenotype collection
- Blood sampling
- DNA extraction and SNP Genotyping
- GWAS Workflow

Sampling locations

Sampling Locations in Greece
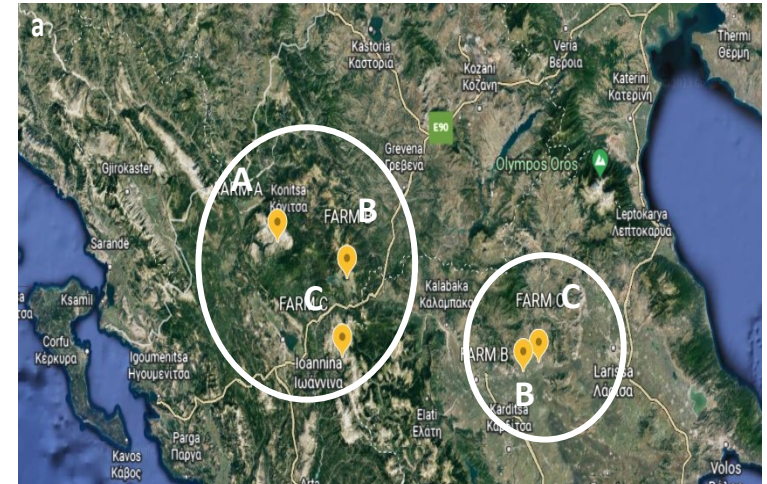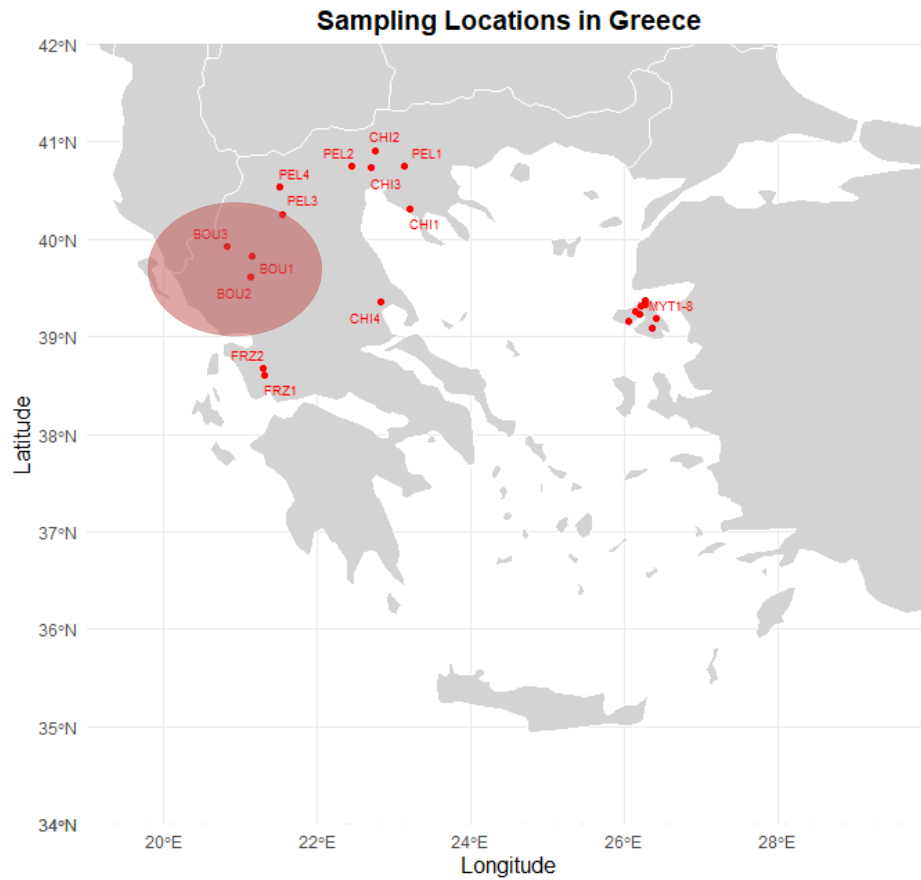
SNP Genotyping across genome
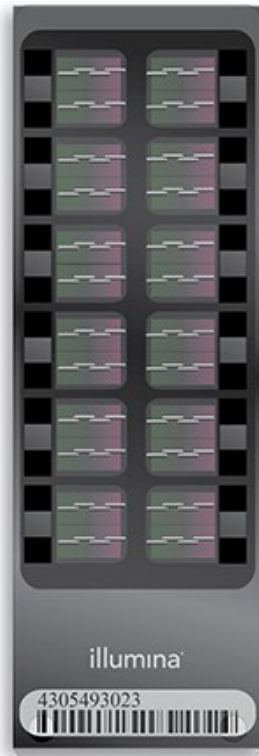
- Blood sample collection

- DNA extraction with Qiagen MiniBlood Kit

Illumina Ovine SNP50 BeadChip – 53,516 SNPs
(https://www.illumina.com/documents/products/datasheets/datasheet_ovinesnp50.pdf)
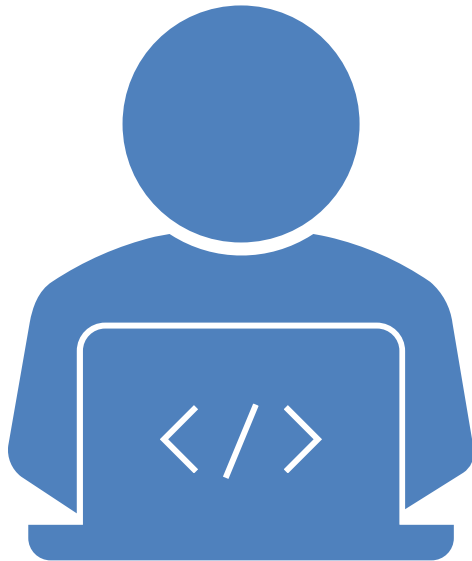
Ped file

map file

binary format

.fam/.bed/.bim

GWAS Workflow

1. Data filtering

2. Assessment of population stratification

3. Association testing

4. Significance testing at genome-wide and chromosome level

Software and Tools

- PLINK v1.9

- GEMMA

- R Studio

Quality Control

Standard filtering steps using plink

- Exclude non-autosomal and unmapped SNPs

- SNP call rate

- Minor allele frequency

- Individual call rate

Genome-wide Association Study (GWAS)

GWAS with GEMMA

Farm
Distance
Speed
elevation gain

Linear mixed model

Continuous trait

Construction of GRM

File with fixed effects and covariates
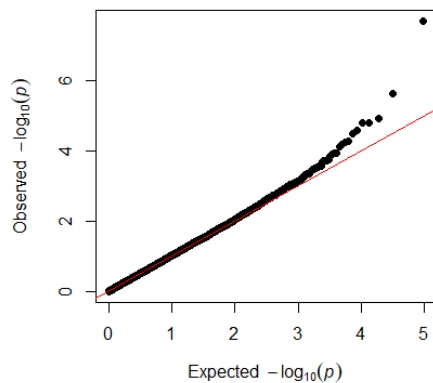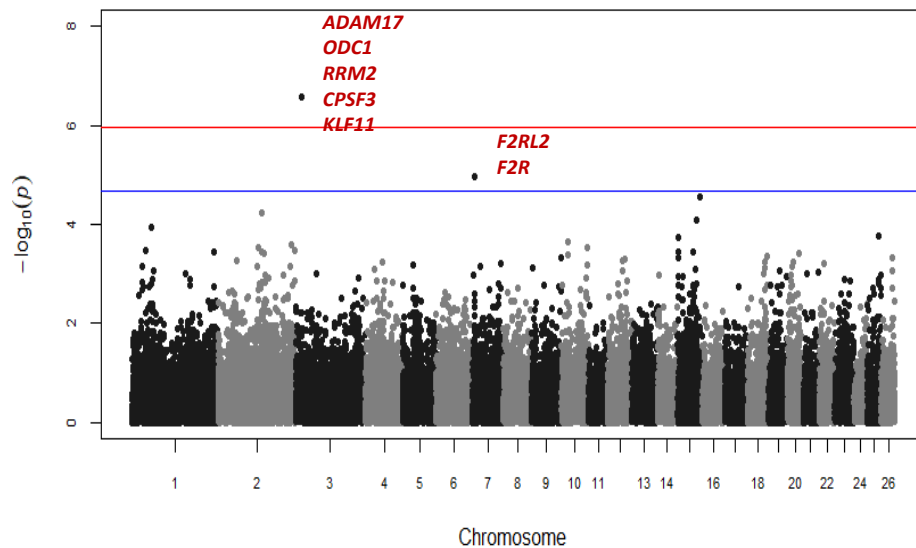
Filtered genotypes with phenotype information

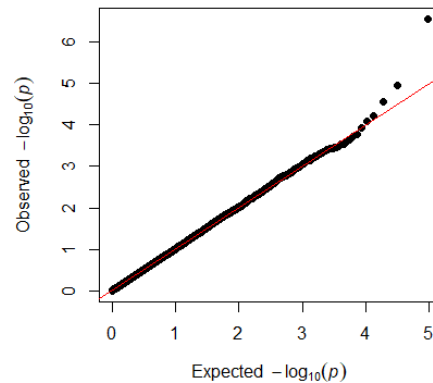# GWAS results – genome-wide Visualization in RStudio

Manhattan plots

QQ plots

GWAS results – chromosome-wide Visualization in RStudio

Daily activity

Altitude difference
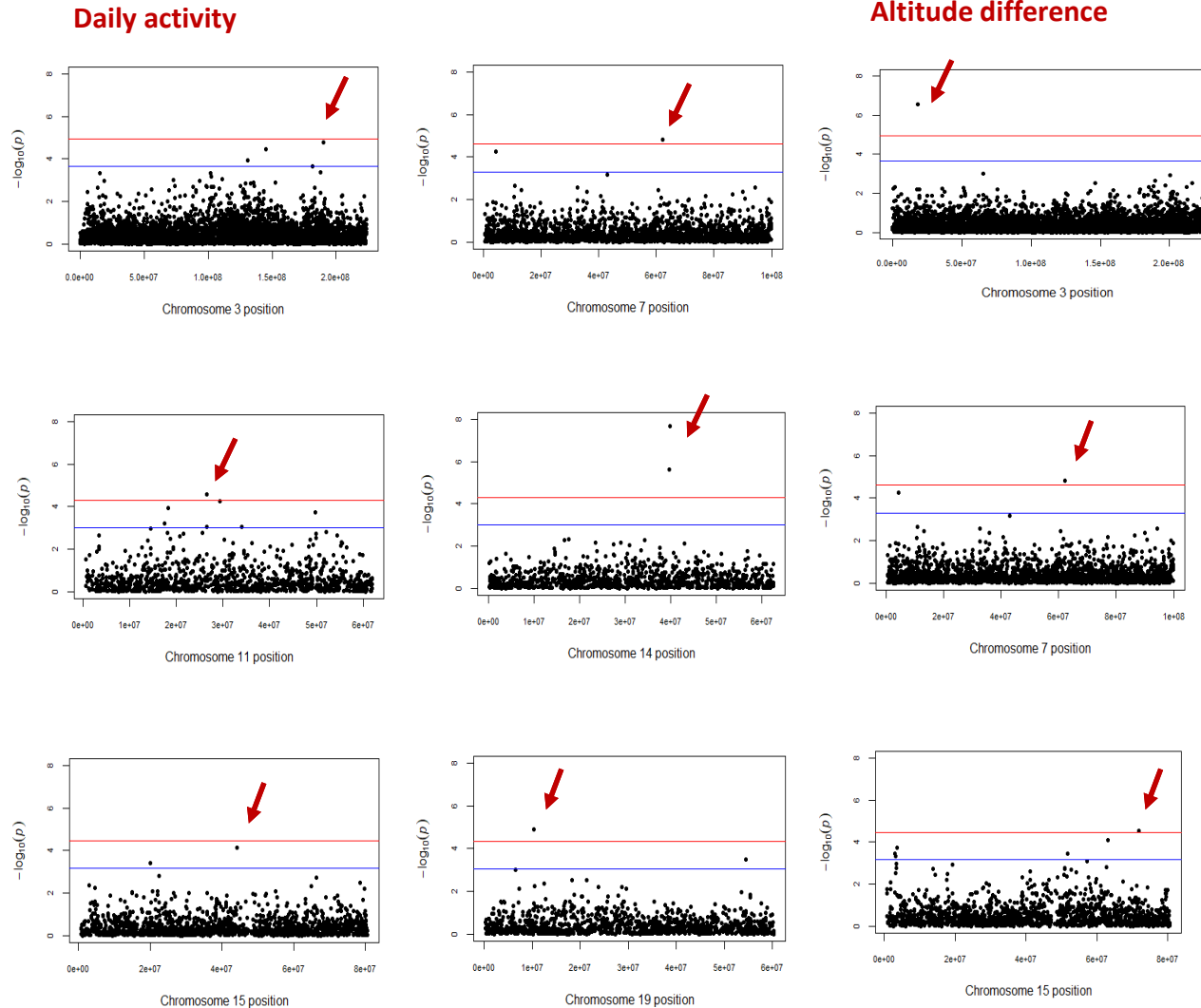
PCA results – Visualization in RStudio
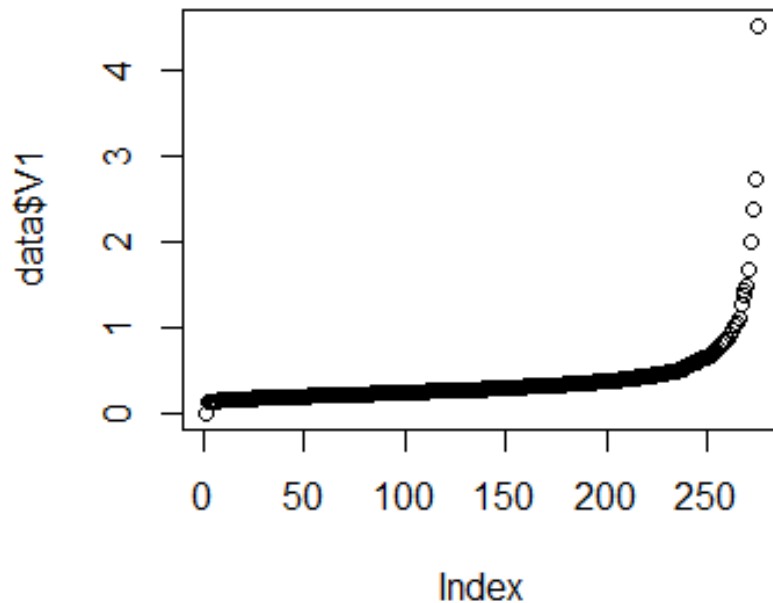
eigen values = variance explained by each PC
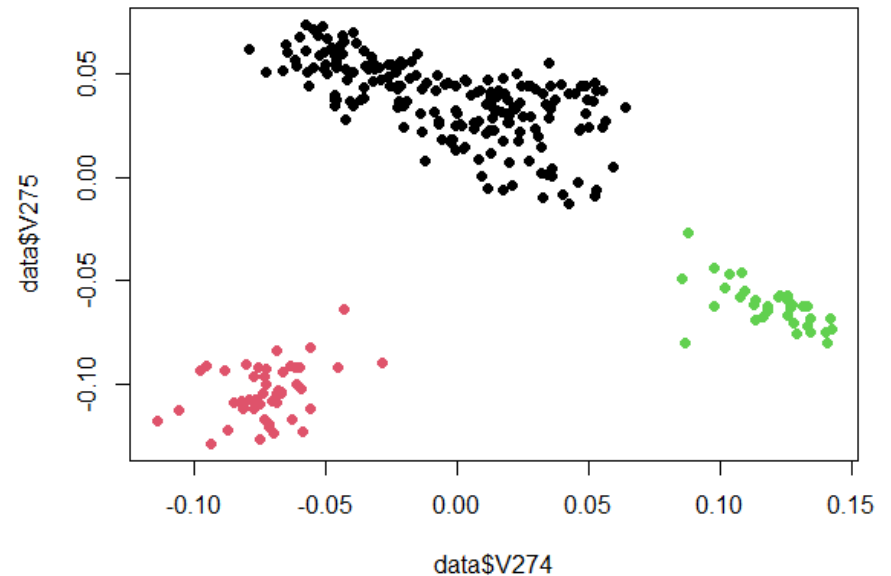
eigen vectors = principal components = directions of data variation

https://github.com/valentinatsar/GWAS/tree/main

Thank you for your attention