

# Biostatistics

Applications in Genetics, Genomics, and other 'omics data

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# Syllabus

## 1. General review

- a. What is Biostatistics?
- b. Population/Sample/Sample size
- c. Type of Data – quantitative and qualitative variables
- d. Common probability distributions
- e. Work example – Malaria in Tanzania

## 2. Applications in Medicine

- a. Construction and analysis of diagnostic tools – Binomial distribution, sensitivity, specificity, ROC curve, Rogal-Gladen estimator
- b. Estimation of treatment effects - generalized linear models
- c. Survival analysis - Kaplan-Meier curve, log-rank test, Cox's proportional hazards model

## 3. Applications in Genetics, Genomics, and other 'omics data

- a. Genetic association studies – Hardy-Weinberg test, homozygosity, minor allele frequencies, additive model, multiple testing correction
- b. Methylation association studies – M versus beta values, estimation of biological age
- c. Gene expression studies based on RNA-seq experiments – Tests based on Poisson and Negative-Binomial

## 4. Other Topics

- a. Estimation of Species diversity – Diversity indexes, Poisson mixture models
- b. Serological analysis – Gaussian (skew-normal) mixture models
- c. Advanced sample size and power calculations

# Application of Fisher's infinite models to binary traits

Anaemia

Dwarfism (?)

Diabetes

Haemoglobin level (Hb)

Height (cm)

Fasting glucose

< 130 g/L in men  
<120 g/L in women

< 147cm

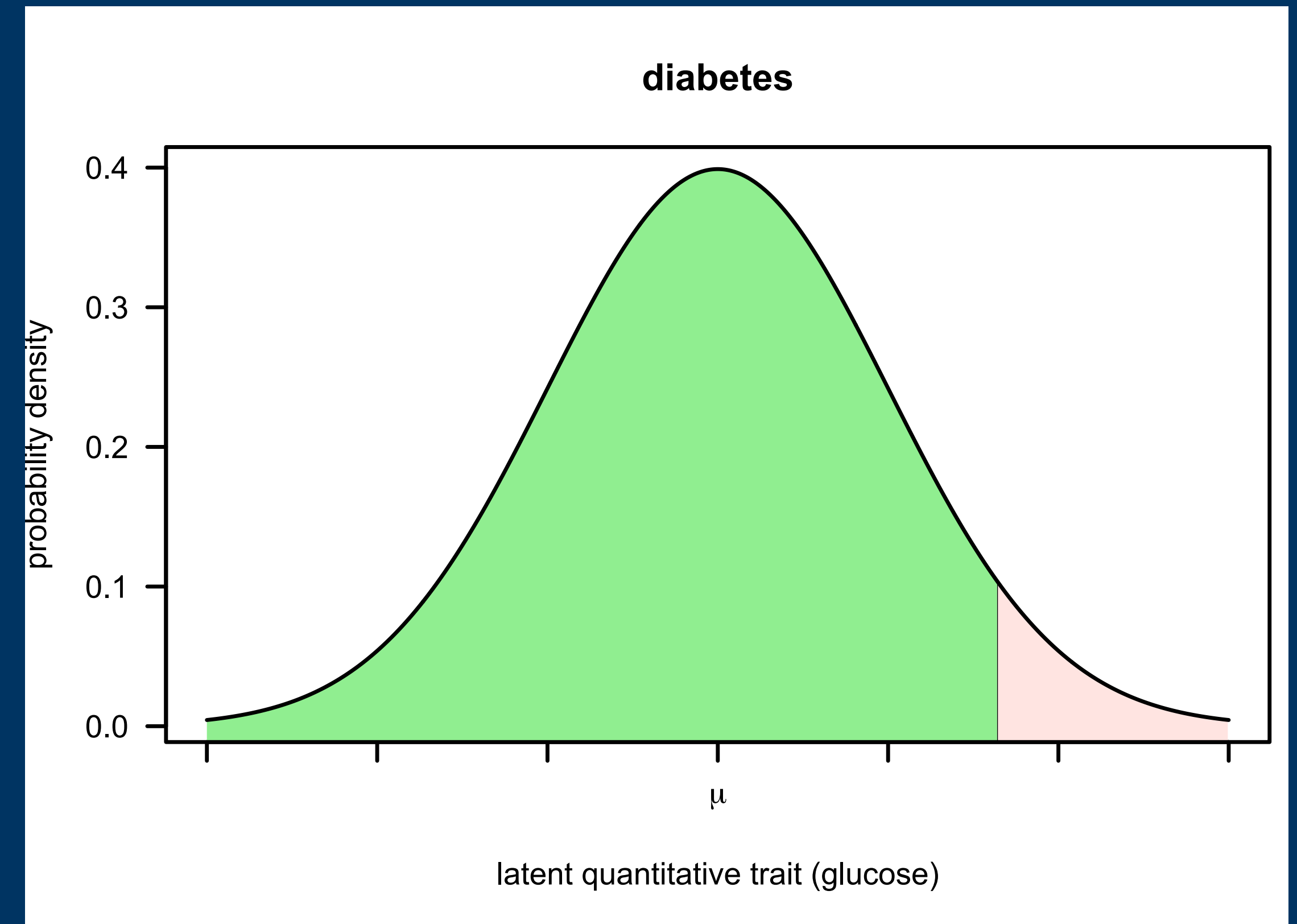
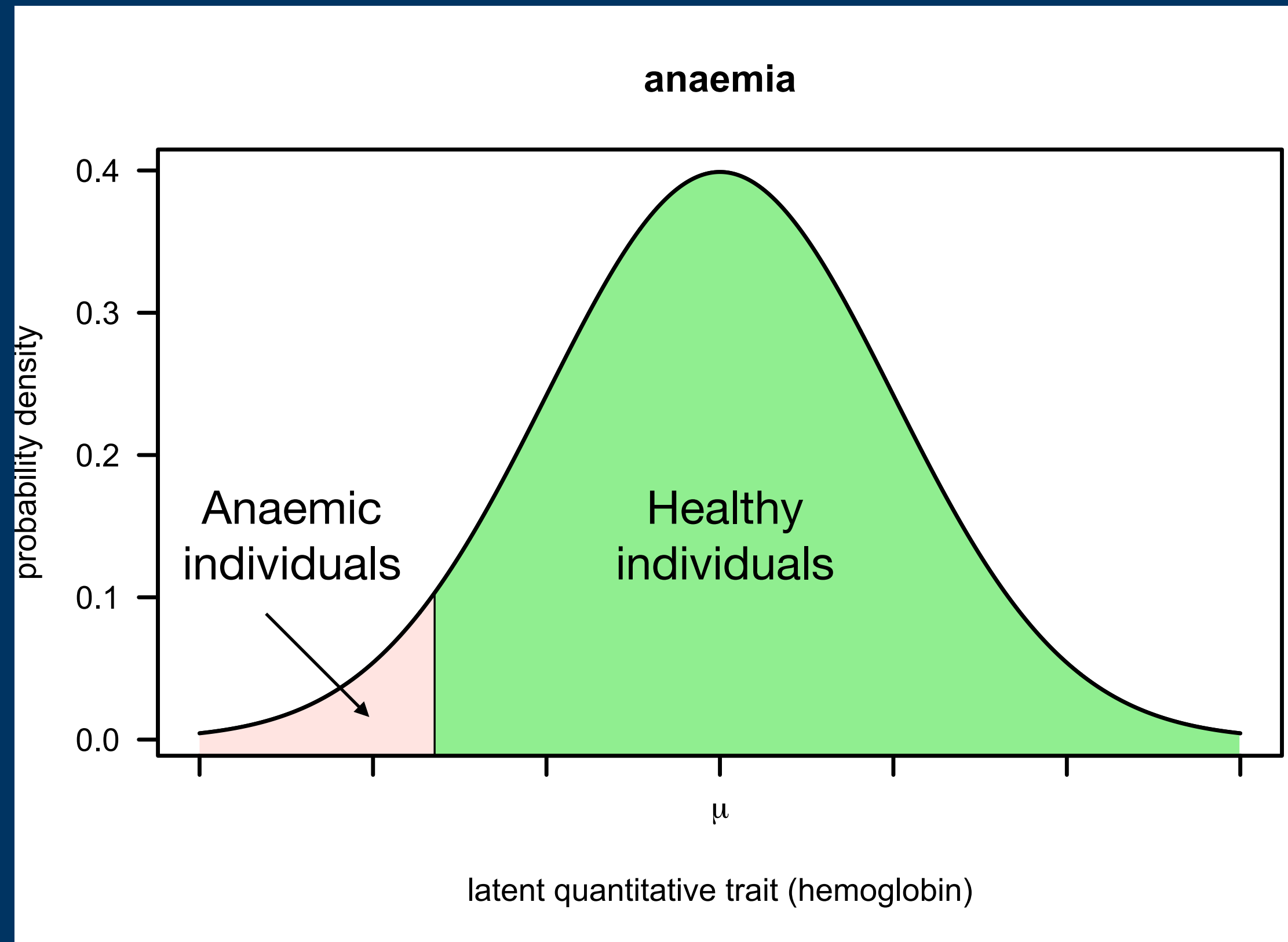
>7.0 mmol/l  
>126 mg/dl

2273 SNPs possible  
associated with Hb

21954 SNPs possibly  
associated with height

405 SNPs possibly  
associated with fasting  
glucose

# Liability models



# Additive probit regression is a liability model

Probit regression

$$\Phi^{-1}(p_i) = \alpha_0 + \alpha_1 X_i \quad X_i \in \{0,1,2\} \quad \text{(single marker)}$$

$$\Phi^{-1}(p_i) = \alpha_0 + \alpha_1 X_i + \beta_1 X_{1i}^* + \dots + \beta_p X_{pi}^* \quad \text{(including other non-generic covariates)}$$

In practice, logistic regression works well (see lecture on GLM)

Probit and logit link functions are only different at the extremes

## Again, testing the effect of a marker on the phenotype

$$H_0 : \alpha_1 = 0 \text{ versus } H_1 : \alpha_1 \neq 0$$

Wald's Score test

Similarly to the additive model for quantitative traits

$$S = \frac{\hat{\alpha}_1}{se(\hat{\alpha}_1)} | H_0 \rightsquigarrow Normal(\mu = 0, \sigma^2 = 1)$$

Wilks' likelihood ratio test

$$\Lambda = (-2) \frac{L(\hat{\alpha}_0^*)}{L(\hat{\alpha}_0, \hat{\alpha}_1)} | H_0 \rightsquigarrow \chi_{(1)}^2$$

$L(\hat{\alpha}_0^*) =$  maximised log-likelihood of the regression model without the covariate

$L(\hat{\alpha}_0, \hat{\alpha}_1) =$  maximised log-likelihood of the regression model with the covariate

## Exercise (probit additive model): data\_Tanzania\_males\_lecture\_10.csv

Assume sampling unrelated individuals

Check information online about rs2289276. Test the association of this genetic marker with anaemia using the probit additive model. Do the same test including age and malaria infection as covariates. Draw your conclusions.

Repeat the above analysis but now for low haemoglobin as the binary phenotype. Draw your conclusions.

## Two main types of studies

Candidate gene association studies

SNPs located in genes known to be  
the biological pathway leading to  
the trait under analysis

10-250 SNPs under analysis

Genome-wide association studies  
(GWAS)

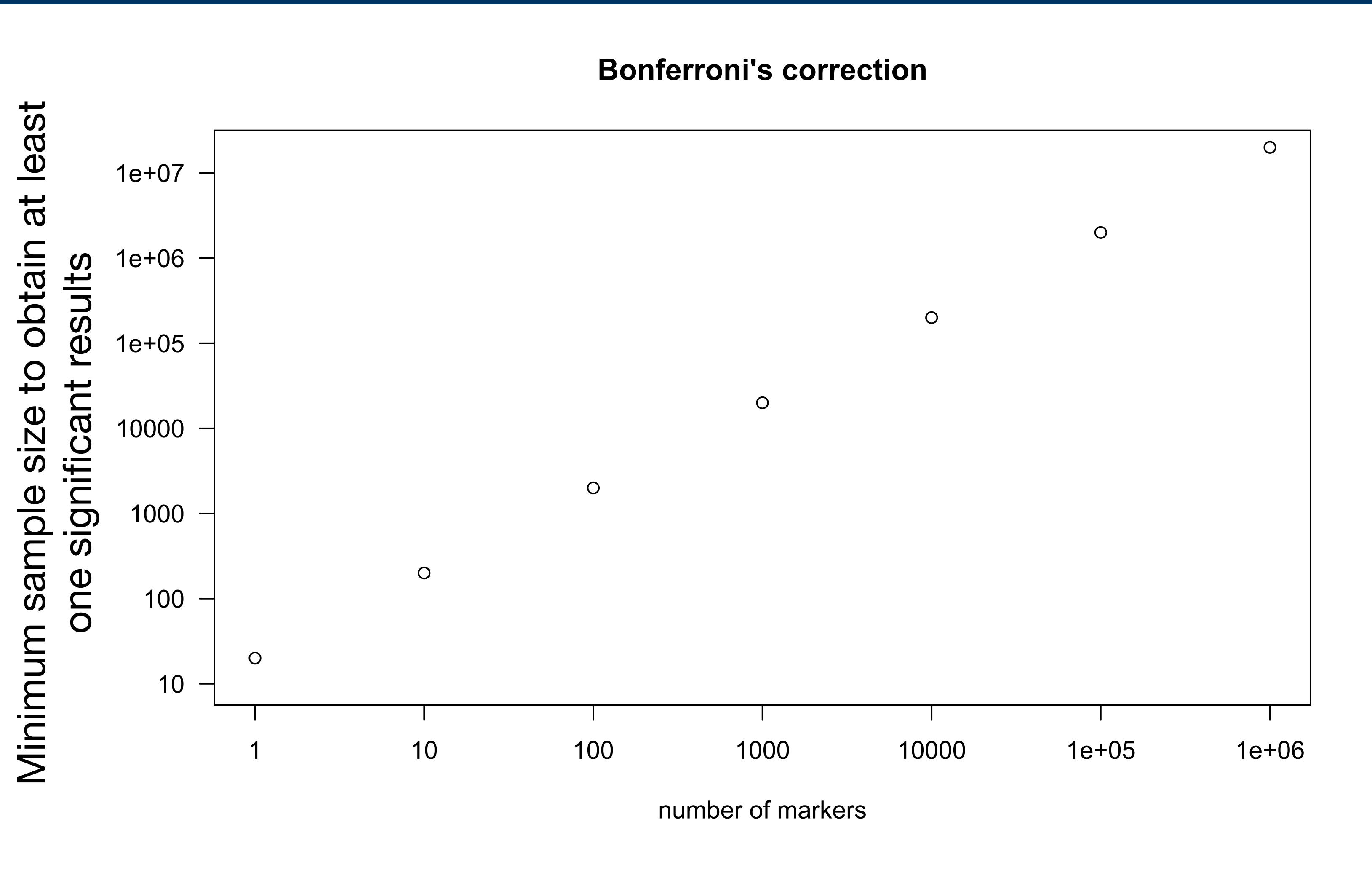
“Fishing expedition”

Millions of SNPs under analysis

What are the practical problems of these studies?



# Practical problems of GWAS



# Global strategy for the analysis

## Candidate gene association studies

Test association between each marker and the phenotype

Additive model

$$\mu_{AA} = \mu + 2\mu_A, \mu_{Aa} = \mu + \mu_A, \text{ and } \mu_{aa} = \mu$$

Dominance/Recessiveness model

$$\mu_{AA} = \mu_{Aa} = \mu + \mu_A, \text{ and } \mu_{aa} = \mu$$

Heterosis model

$$\mu_{AA} = \mu_{aa} = \mu, \mu_{Aa} = \mu + \mu_{AA}$$

General model

$$\mu_{AA}, \mu_{Aa}, \mu_{aa}$$

Report the lowest p-value among all the models tested

Correct significance level for multiple testing (Bonferroni/Sidak-Dunn)

Check the distribution of p-values (deviations from the uniform distribution are evidence for true associations)

# Global strategy for the analysis GWAS

Test association between each marker and the phenotype

Additive model

$$\mu_{AA} = \mu + 2\mu_A, \mu_{Aa} = \mu + \mu_A, \text{ and } \mu_{aa} = \mu$$

Report the p-value for marker tested

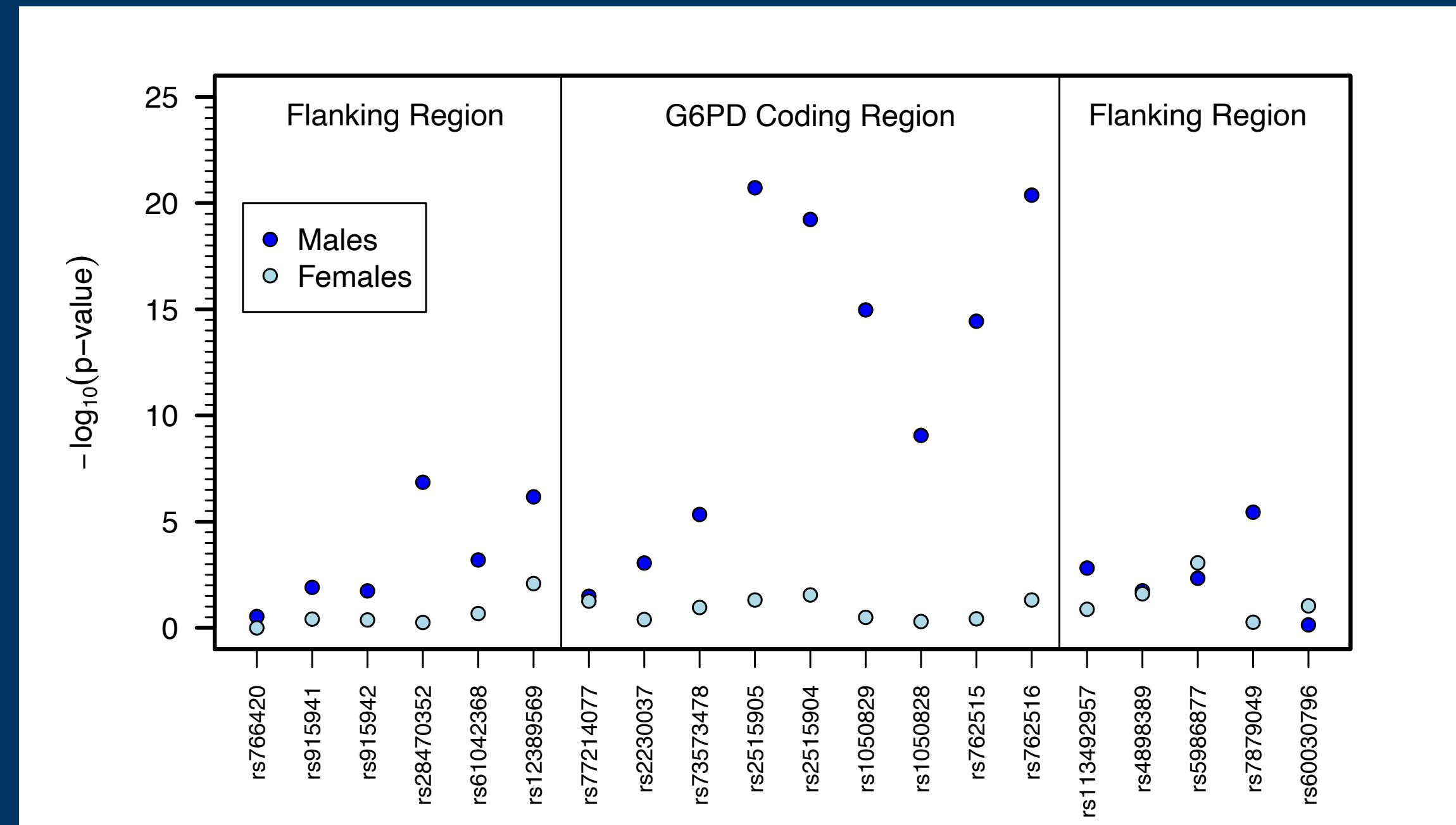
Adjust the p-values for multiple testing

Check the distribution of the p-values as for

Great deal of computational  
efficiency

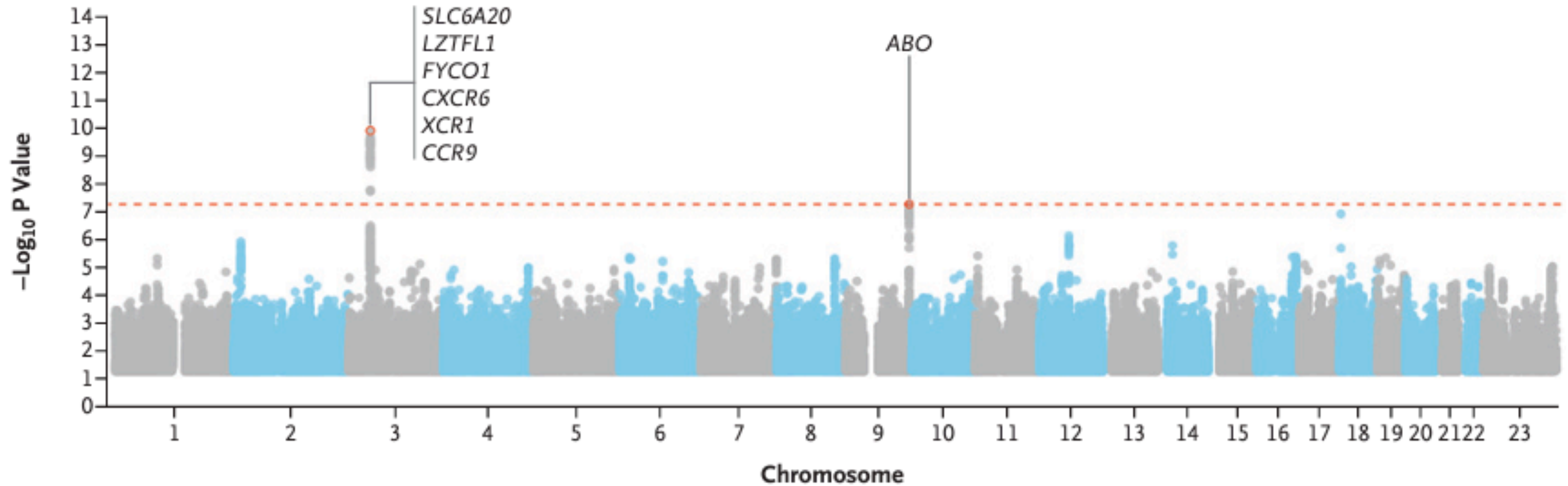
**Note:** GWAS is usually analysed in the standalone PLINK software (not in the software R).

# Main outputs (Candidate gene association study)



# Main outputs - GWAS

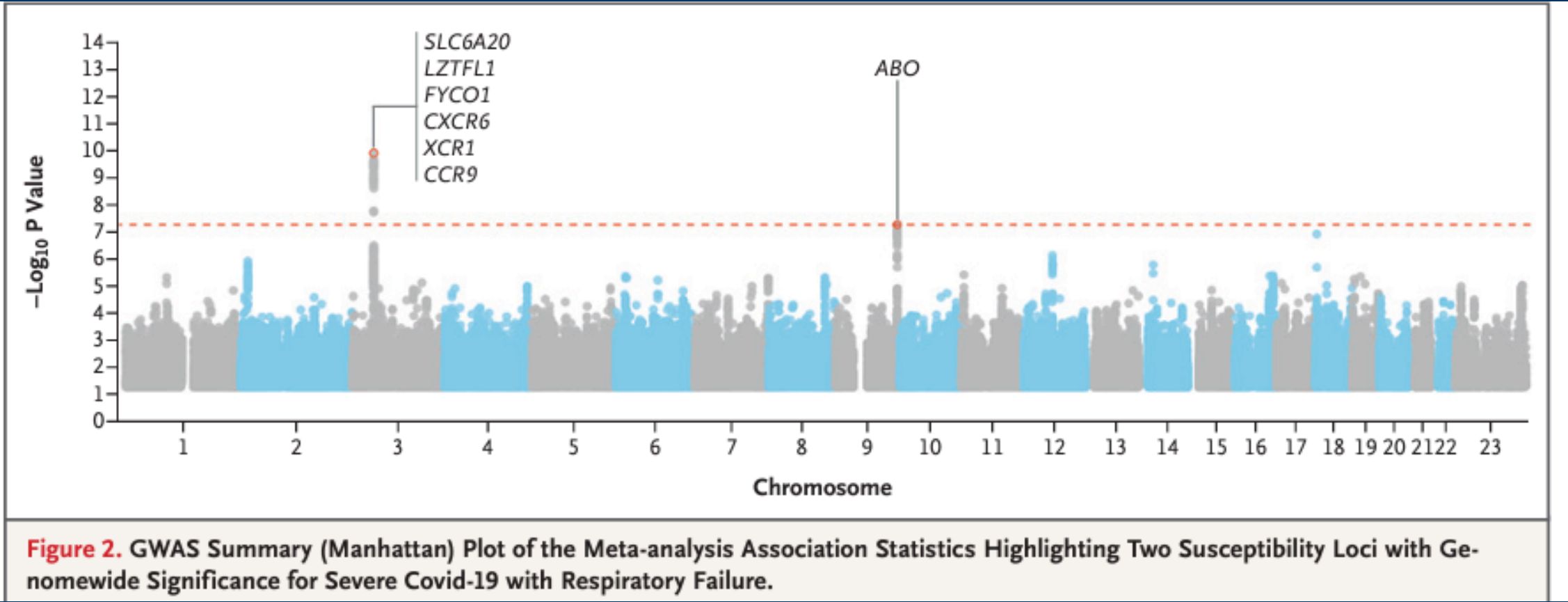
Do you know how this plot is called?



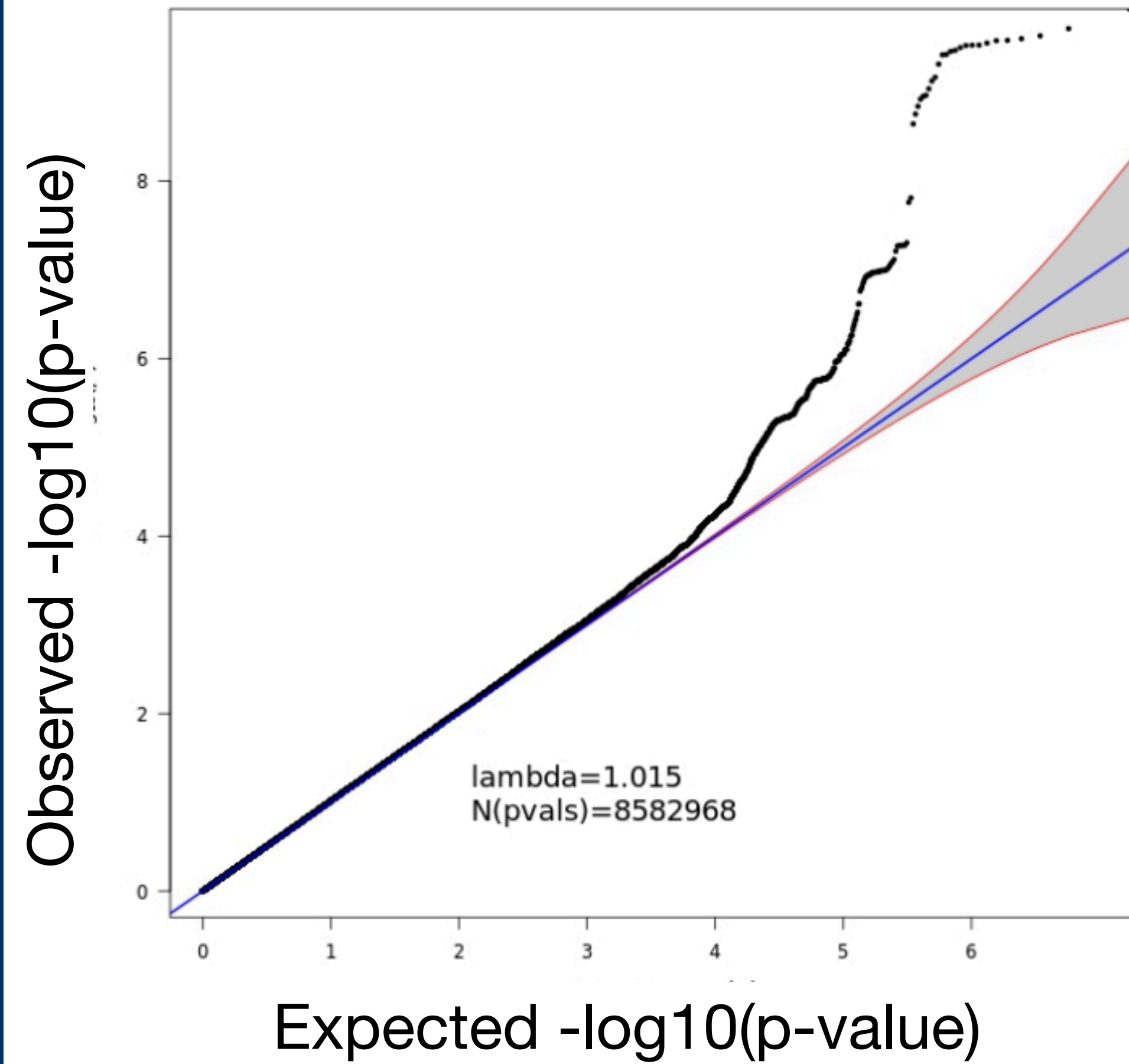
**Figure 2.** GWAS Summary (Manhattan) Plot of the Meta-analysis Association Statistics Highlighting Two Susceptibility Loci with Genomewide Significance for Severe Covid-19 with Respiratory Failure.



# Manhattan plot



# Main outputs - GWAS



## Practical - data\_lecture\_11\_gwas.csv

Let's recreate the typical visual outputs from a GWAS on COVID-19 in the Portuguese population



# Controlling the proportion of true positive among significant results

	True Positive	False Negative	Total
Significant results	m11	m12	m1
Non-significant results	m21	m22	m2
Total	m_1	m_2	m

## False discovery rate (Benjamini-Hochberg (BH) procedure)

$$E [\text{True positives} \mid H_0 \text{ rejected}] = \alpha^*$$

$$E \left[ \frac{m_{11}}{m_{11} + m_{21}} \right] = \frac{q}{\alpha} = \alpha^* \qquad \alpha^* = 0.05$$

## False discovery rate (Benjamini-Hochberg (BH) procedure)

Algorithm (under the assumption of independent tests)

1. Order all the p-values by increasing order,  $p_{(1)}, \dots, p_{(n)}$
2. For  $\alpha^*$ , find  $k$  such as  $p_{(k)} \leq \frac{k}{m}\alpha^*$
3. Reject the null hypothesis (i.e., declare discoveries) for all the genetic markers associated with p-values less  $p_{(k)}$

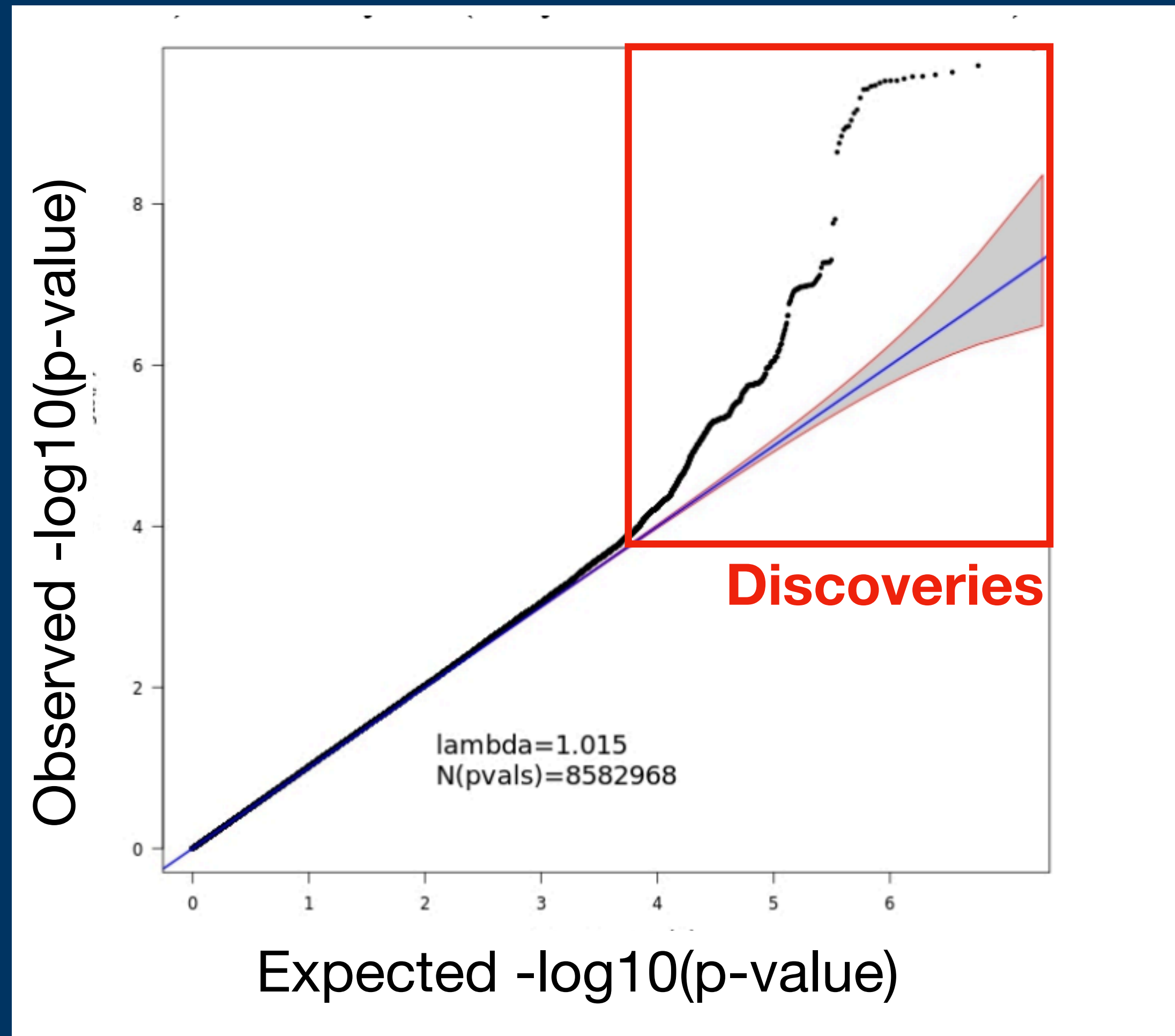
where

$p_{(1)}$  is the minimum p-value (with rank 1)

$p_{(k)}$  is the p-value with rank  $k$

$p_{(n)}$  is the maximum p-value (with rank  $n$ )

# Visual interpretation



## Adjusting p-values

1. Order all the p-values by increasing order
2. Assign the ranking or position to each p-value
3. Calculate the adjusted by  $p_{(i)}^{adj} = \min \left\{ 1, \min_{j \geq i} \frac{mp_{(j)}}{j} \right\}$

where

$p_{(i)}^{adj}$  is the adjusted p-value with rank  $i$

$p_{(j)}$  is the p-value with rank  $j$

$m$  is the number of tests

Reject null hypothesis of tests whose the adjusted p-values are below the FDR

# Useful variants of Benjamini-Hochberg

## **Benjamini-Yekutieli (BY) procedure for dependent tests**

Adequate when analysing data from genetic markers in the same genetic locus

## **Benjamini-Krieger-Yekutieli (BKY) (improved) procedure for independent tests**

Package mutoss has implementations of these and other procedures

Package MASS has implementations of the BH and BY procedures

## Exercise - data\_lecture\_11\_gwas.csv

Apply Benjamini-Hochberg and Benjamini-Krieger-Yekutieli procedures to p-values from genetic markers in chromosome 10

How many genetic markers are statistically significant according to these procedures?