Lecture 3 : Statistics for Human Geneticists

Volos Summer School

21 / 05 / 2018

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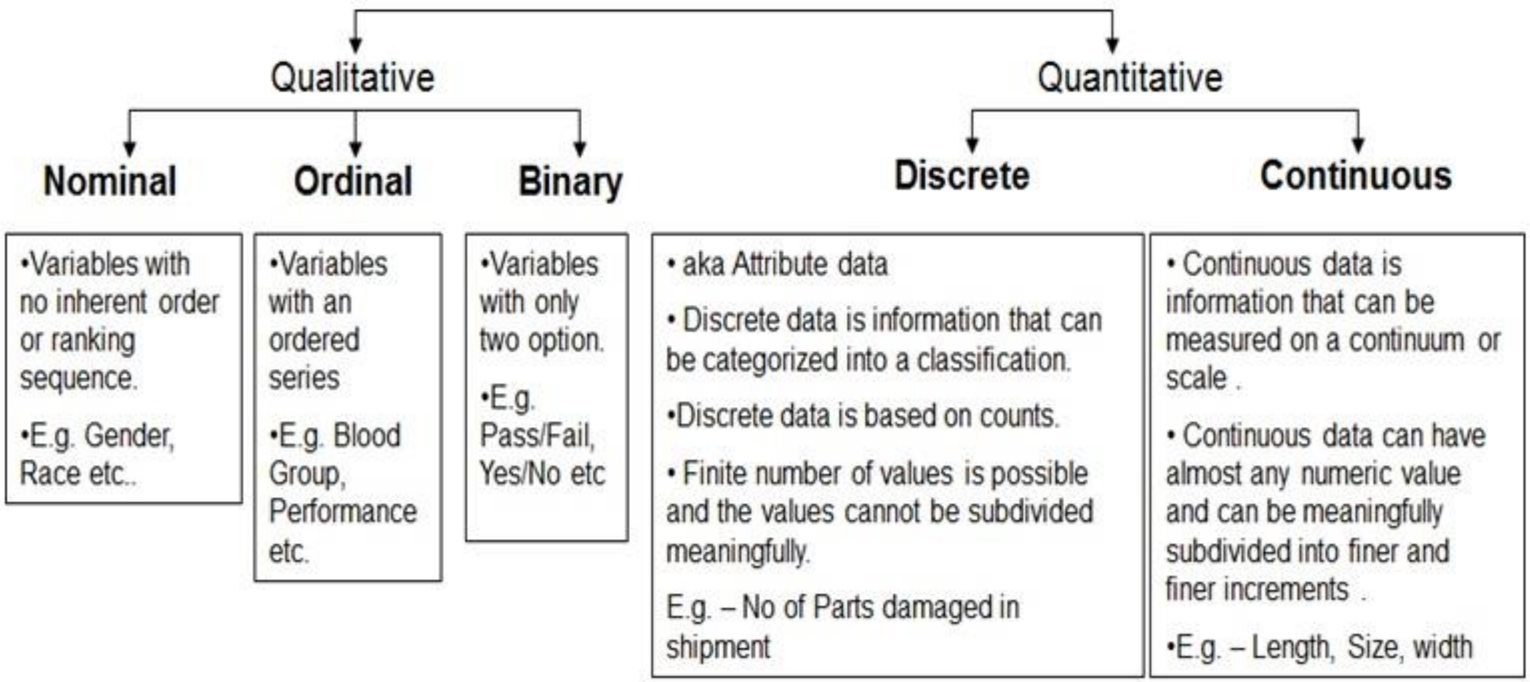
What can we do with statistics?

- Estimation
- Hypothesis testing
- Modelling
- Predicting

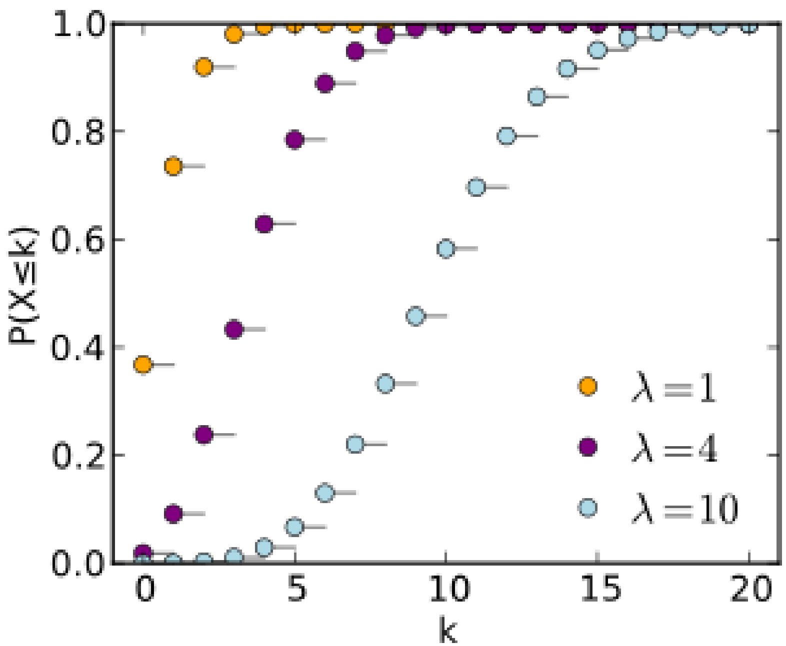
Random Variables

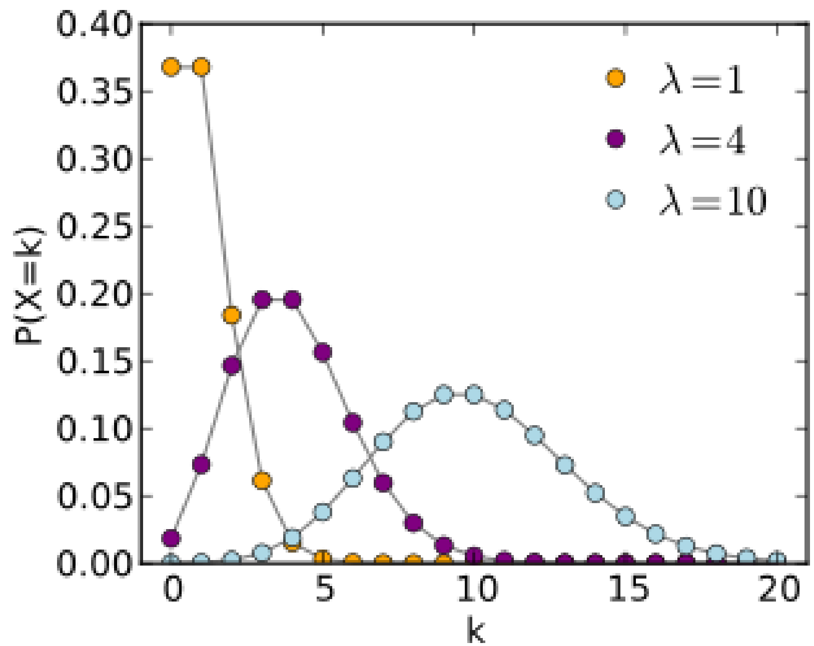
- In statistics, we measure realizations of random variables
- Often, random variables follow a distribution
- They can be qualitative or quantitative, continuous or discrete



Distributions

- Two ways to represent them :





Cumulative distribution function (CDF)

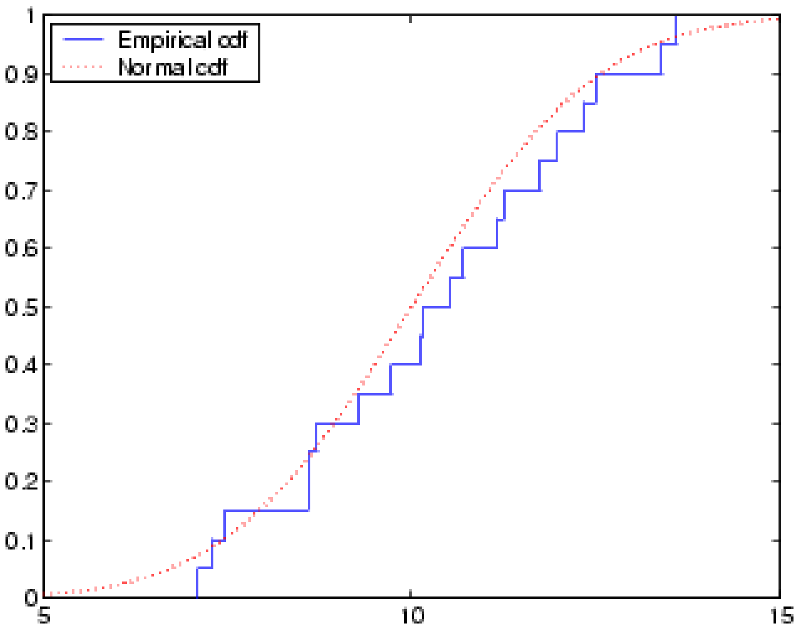
- $y = p(X \leq x)$
- Always growing
- Ideal way to represent, but hard to read
- All distributions look the same

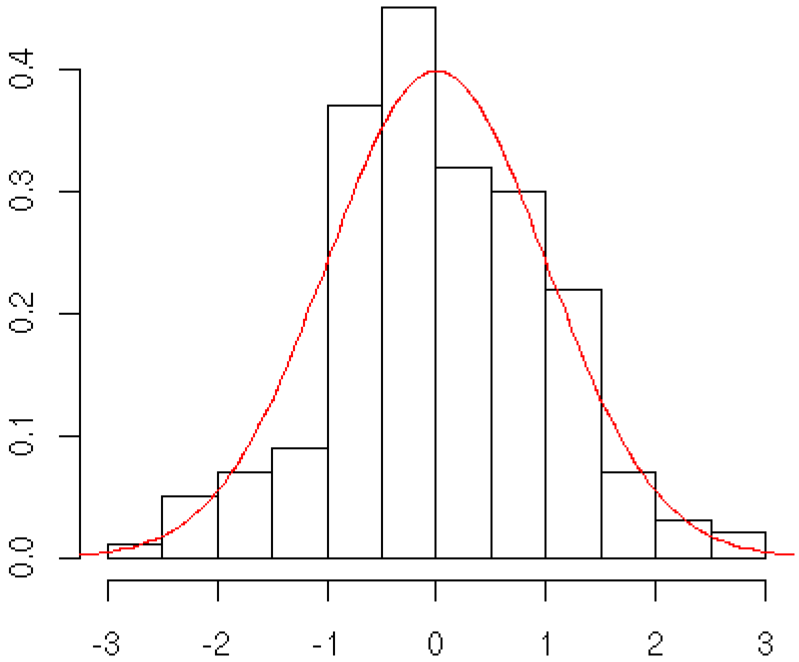
Probability density function (PDF)

- $y = p(X = x)$ for discrete
- Shows how values are distributed
- Nice visually, but mathematically hard to deal with

Distributions

- How to estimate them:





Empirical CDF

- Rarely used
- Except when you want to compute empirical quantiles

Barplot (discrete)

- For every value, count occurrences

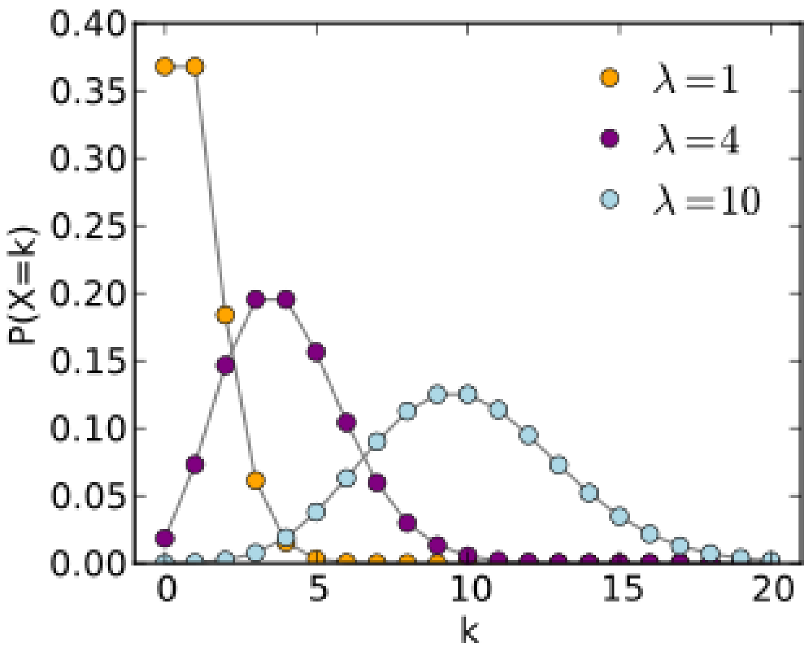
Histogram (continuous)

- Cut the interval into bins, count observations within bin

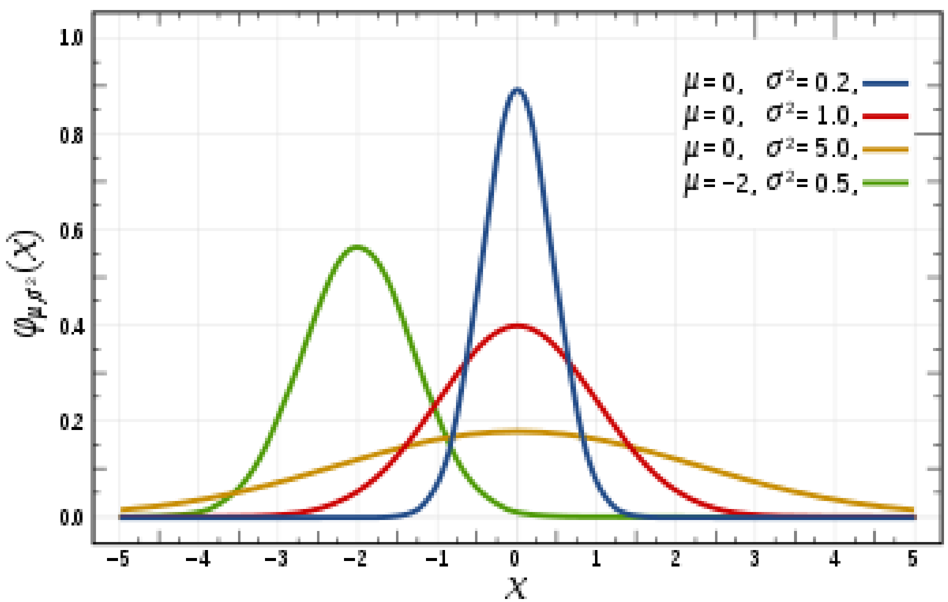
Data input

Distributions

- Two broad types:
  - those followed by random variables (real world data)



$X \sim Poisson(\lambda)$



$X \sim \mathcal{N}(\mu, \sigma^2)$

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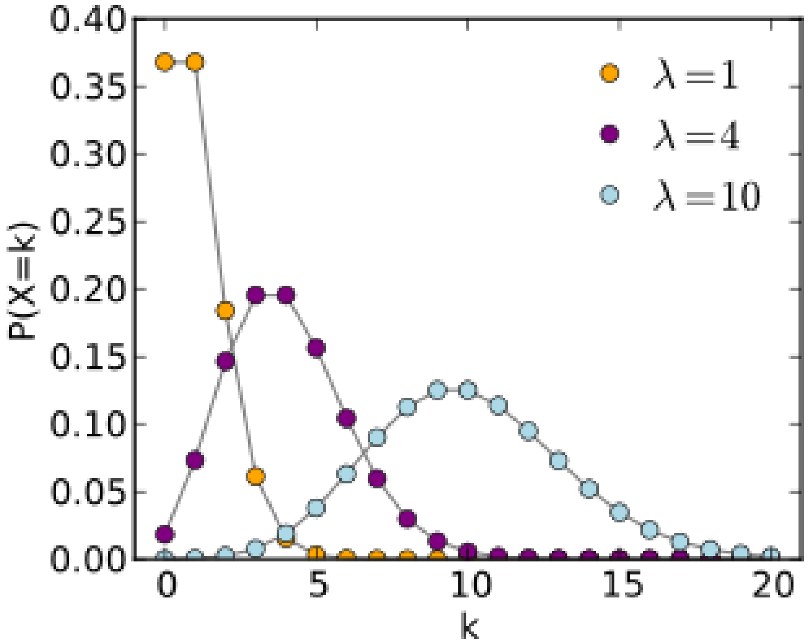

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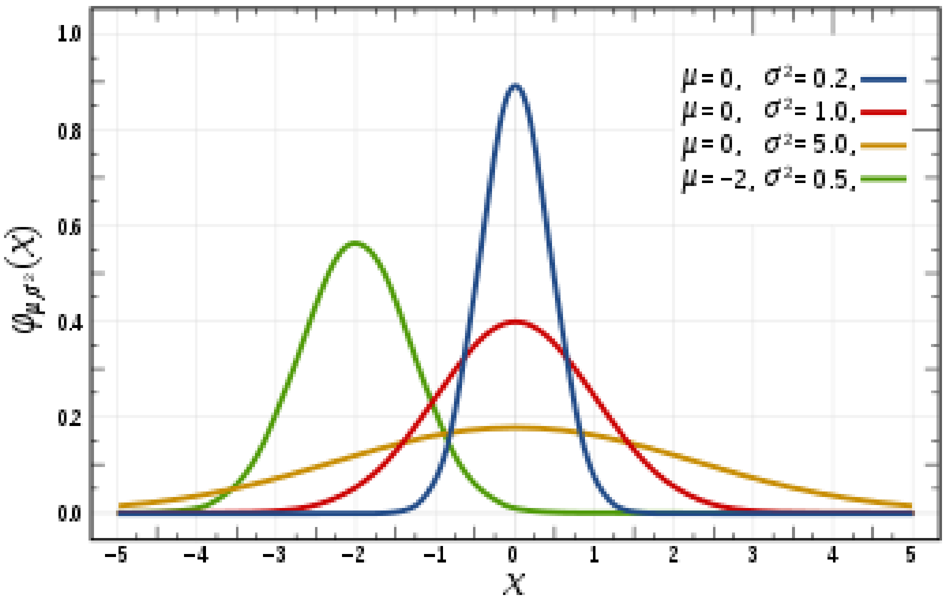

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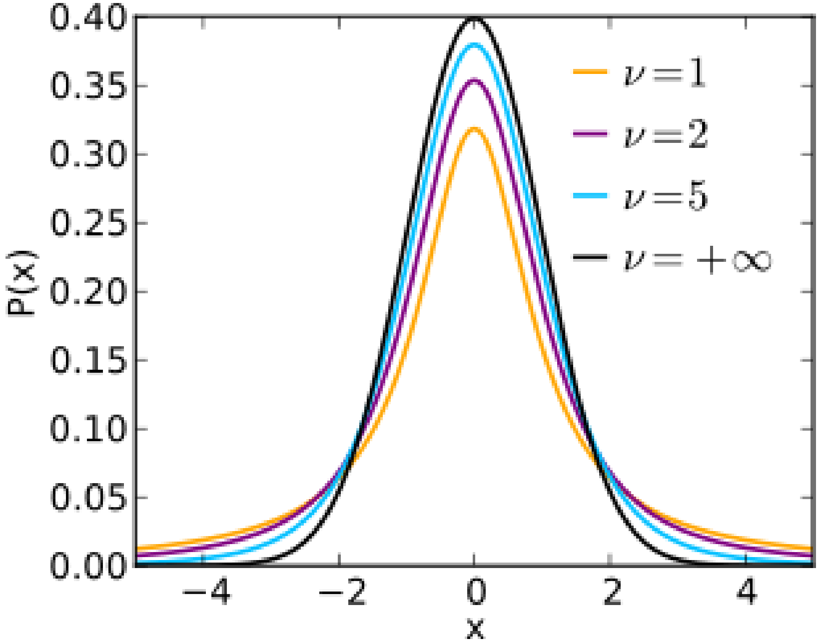
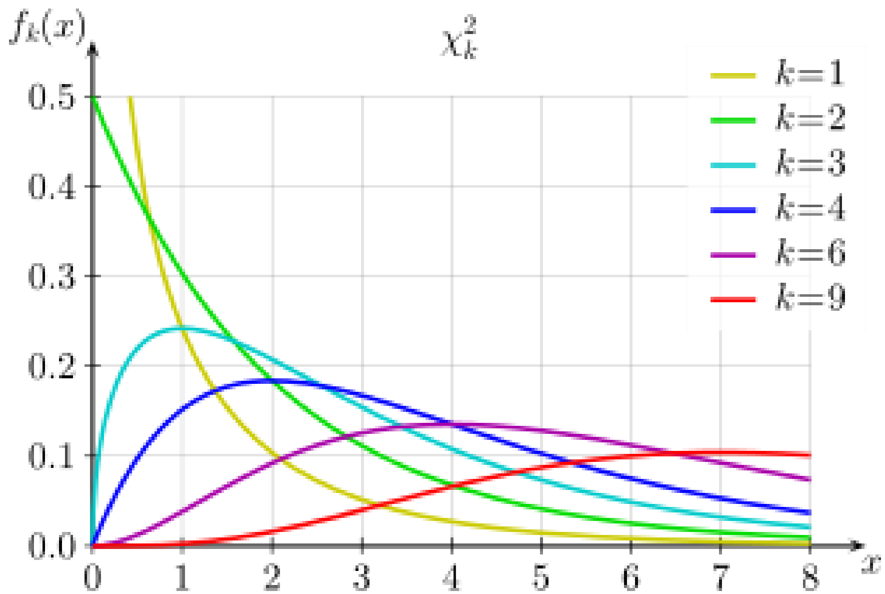
Distributions

- Two broad types:
  - those followed by test statistics



$X \sim T(\nu)$



$X \sim \chi^2(k)$

λ, μ, σ, ν and k are ideal parameters. How to estimate them?

Statistics

- A statistic is a meaningful quantity derived from the data
- Often, estimators are realization of distribution parameters
- Examples? Mean, proportion
- For simple distributions/parameters, there is a formula
- For more complex ones, we have to use other techniques (Monte-Carlo, Permutations…)

$$(\hat{\mu} =)\ \bar{x} = \frac{1}{n}\sum_{k=0}^{n} x_k$$

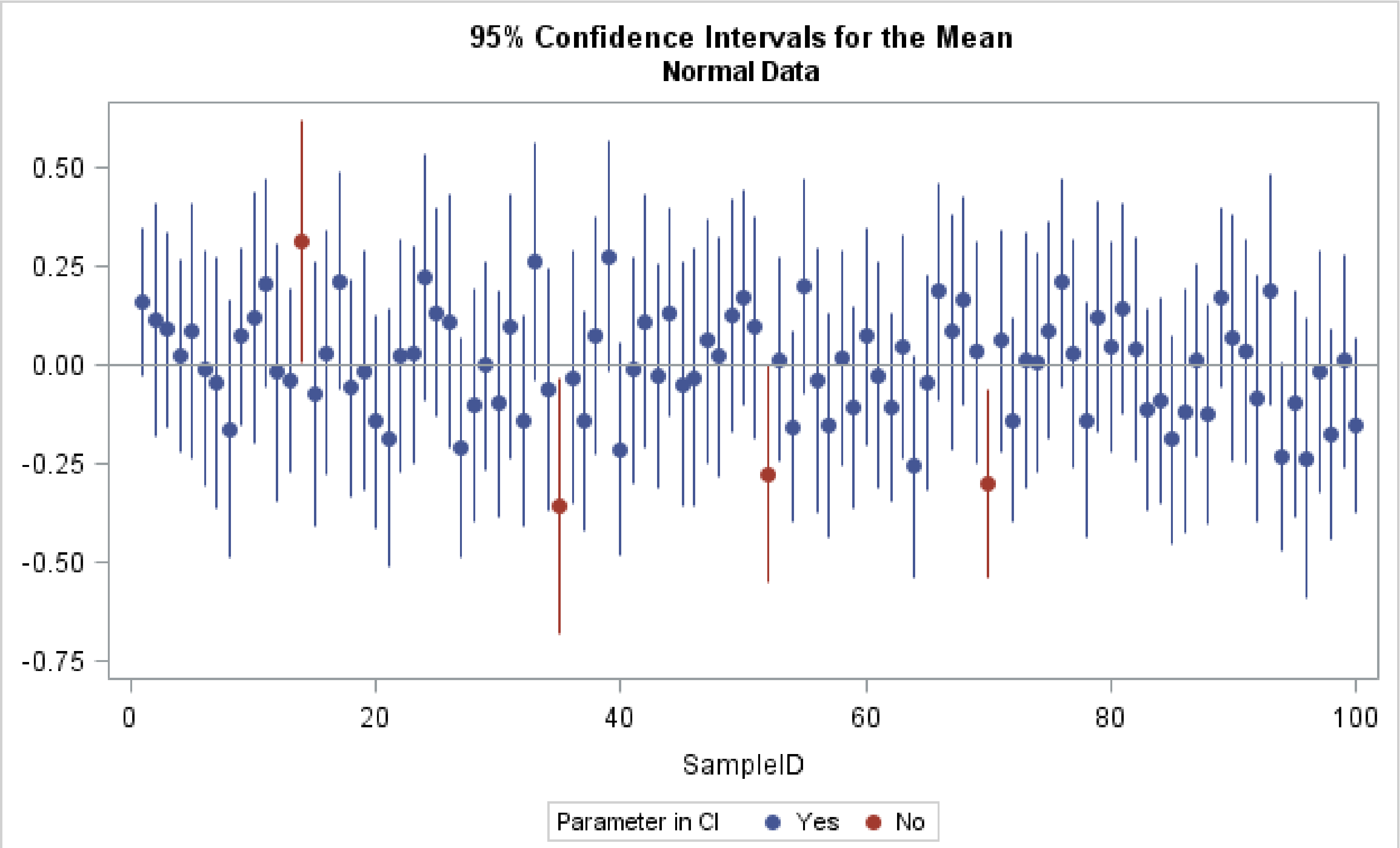
$$\hat{p} = \frac{x}{n}$$

$$w = \frac{\left(\widehat{\theta} - \theta_0\right)^2}{se(\widehat{\theta})} \sim \mathcal{N}(0,1)$$

$$\left(\widehat{\sigma^2} =\right) s^2 = \frac{1}{N-1}$$

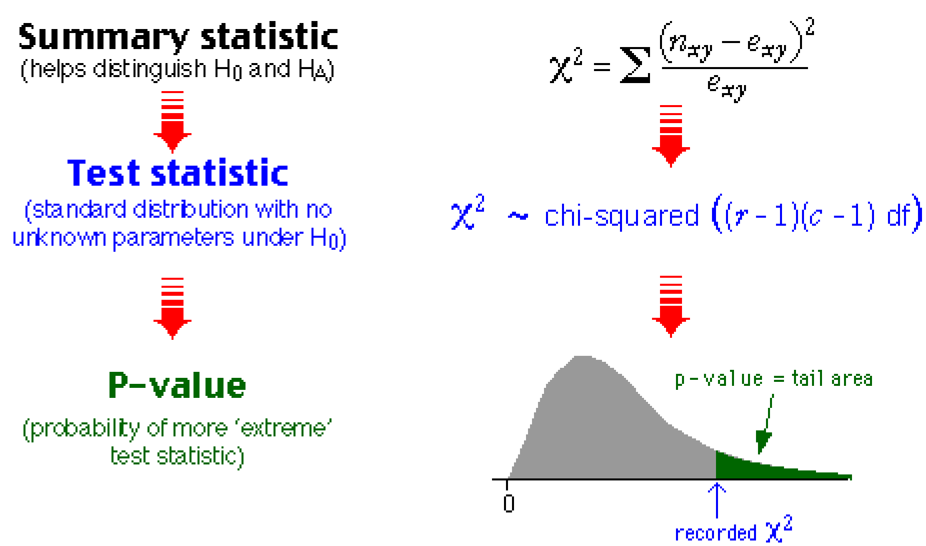
One particular statistic: Confidence intervals

- x% confidence interval (x%C.I.) : x% of the time when this interval is calculated, it will contain the true value of the parameter



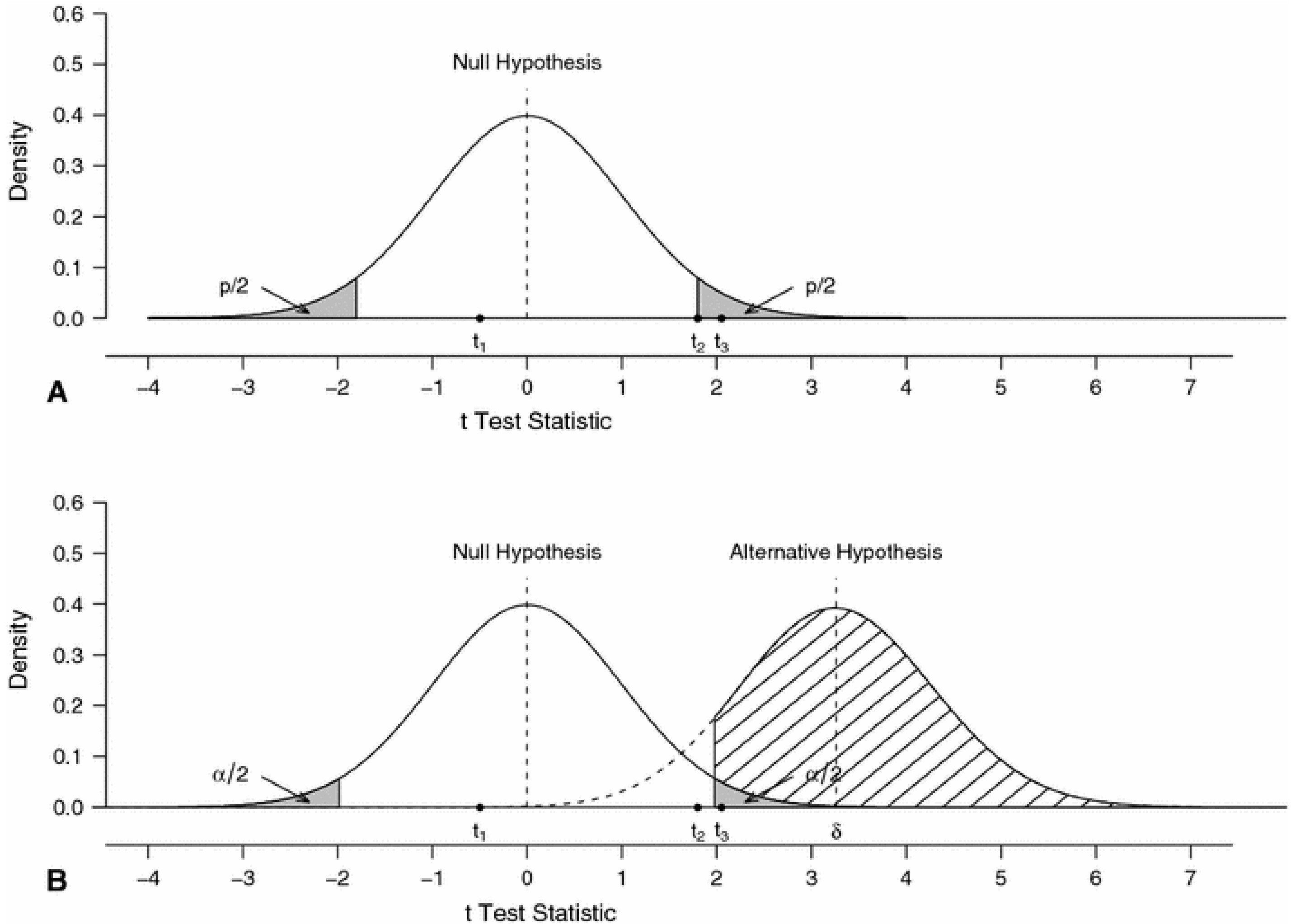
Hypothesis testing

- We want to measure whether the data gives sufficient evidence to reject a hypothesis
- Null/Alternative hypothesis ($\mathcal{H}_0/\mathcal{H}_A$)
- We prove that we can produce a statistic that follows a certain distribution if the null hypothesis is true = name of the test
- We calculate the statistic based on our data
- Because we know the distribution, we can calculate the CDF $p(X \leq x)$
- = how unlikely it is that our measurement comes from the null : p-value
- Example: proportion test, t-test, chi-squared test…



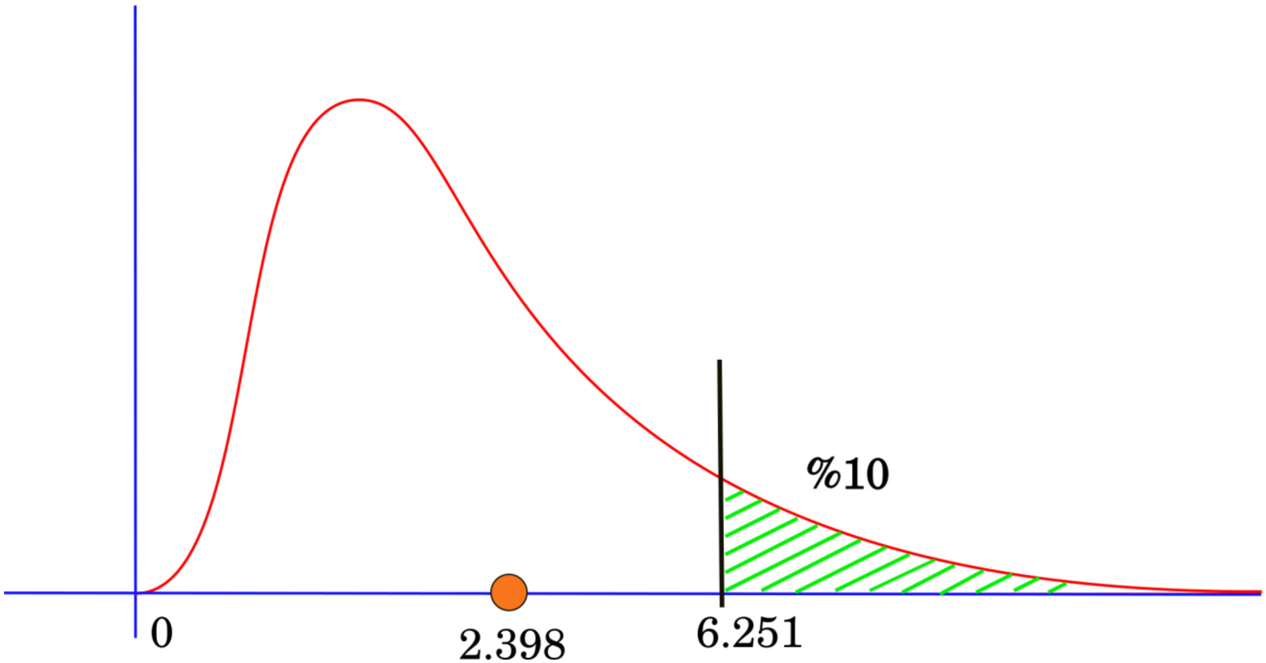
Data input

Hypothesis testing



Data input

Two tails or one



Exercise 1 : Proportion test

- In a population, we observe 41,009 potentially damaging variants among 14,281,180 variants
- What is the proportion? In a very large reference population, we observe a proportion of 1.52×10^{-3}. Is it significantly different? (prop.test, binom.test)

Modelling and predicting

- If we estimate the effect of one variable on another variable, we do modelling
- When we apply this effect to new observations of the variable, we do prediction
- Process is called machine learning, predictive modelling or predictive analysis
- In human genetics, main task is to model effect of genotypes on phenotypes

$$\boldsymbol{phenotype} \sim \boldsymbol{\beta} \times \boldsymbol{genotype} + \boldsymbol{\epsilon}$$

$$\begin{bmatrix} pheno_0 \\ \vdots \\ pheno_n \end{bmatrix} \qquad \begin{bmatrix} A/T \\ \vdots \\ T/T \end{bmatrix}$$

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$= \{0,1\}$ (case-control)

$\in \mathbb{R}$ (quantitative) $\sim\mathcal{N}(0,1)$

$= \{0,1,2\}$ (genotype, directly typed

$\in [0,2]$ (dosage, imputed) $\begin{bmatrix} 0.965 \\ \vdots \\ 1.816 \end{bmatrix}$

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Usually, we do not predict (except PRS)

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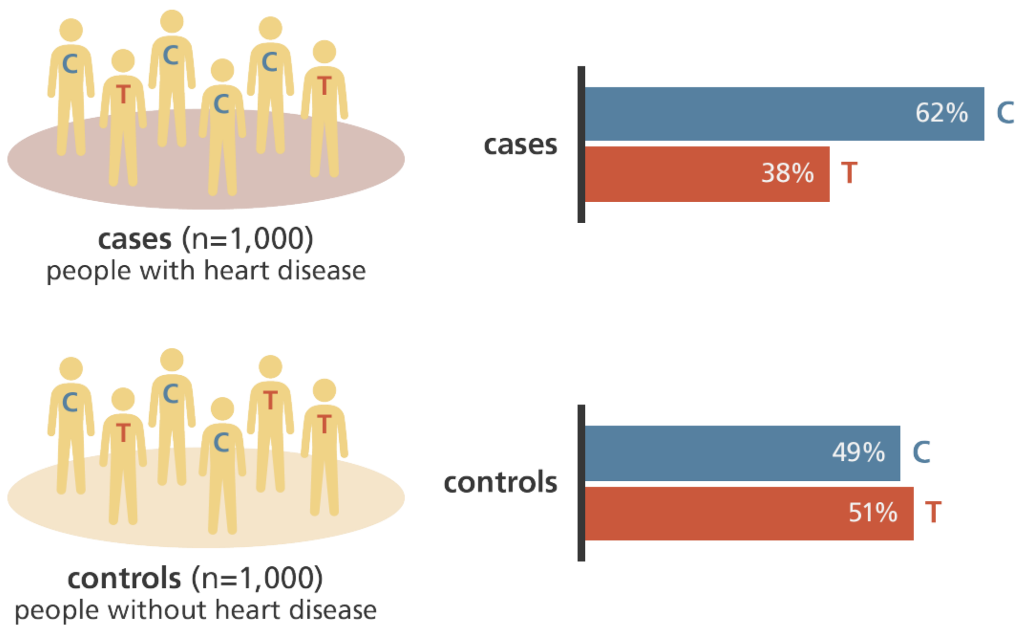
Data input

Case/control

- Estimated effect: odds ratio (OR)

  "how much more likely are you to be a case if you carry the risk allele?"

  per genotype, calculate the odds $O = \frac{p}{1-p}$



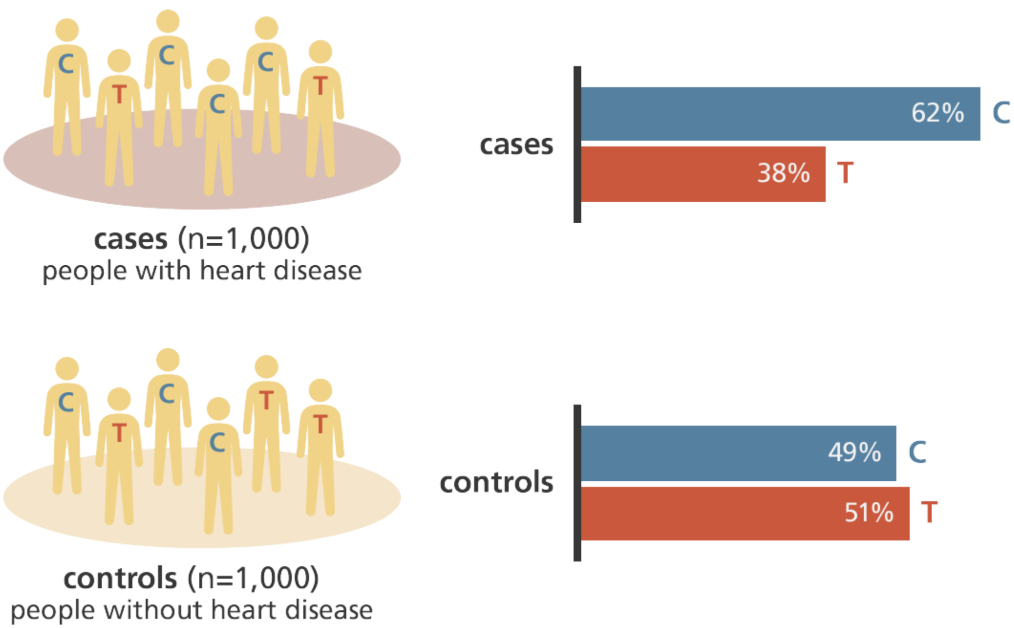
Data input

Case/control

- Estimated effect: odds ratio (OR)

  "how much more likely are you to be a case if you carry the risk allele?"

  per genotype g and for a disease Y, calculate the odds $O = \frac{p_{Y=1|g}}{1-p_{Y=1|g}}$



	cases	controls
T	380	490
C	620	510

$$O_T = \frac{380/n_T}{490/n_T} \qquad O_C = \frac{620/n_C}{510/n_C}$$

$$OR_{C/T} = \frac{620 \times 490}{510 \times 380} = 1.56$$

Data input

Case/control

Dominant

Marker allele	Affected	Unaffected
DD+Dd	$n_{2A} + n_{1A}$	$n_{2U} + n_{1U}$
dd	n_{0A}	n_{0U}

$$OR = \frac{n_{affected\ carriers} \times n_{healthy\ non-carriers}}{n_{healthy\ carriers} \times n_{affected\ non-carriers}}$$

Recessive

Marker allele	Affected	Unaffected
DD	n_{2A}	n_{2U}
Dd+dd	$n_{1A} + n_{0A}$	$n_{1U} + n_{0U}$

Additive

Marker genotype	Affected	Unaffected
DD	n_{2A}	n_{2U}
Dd	n_{1A}	n_{1U}
dd	n_{0A}	n_{0U}

$$\mathrm{OR} = \frac{(2 \times n_{2A} + n_{1A}) \times (2 \times n_{0U} + n_{1U})}{(2 \times n_{0A} + n_{1A}) \times (2 \times n_{2U} + n_{1U})}$$

Allelic odds-ratio

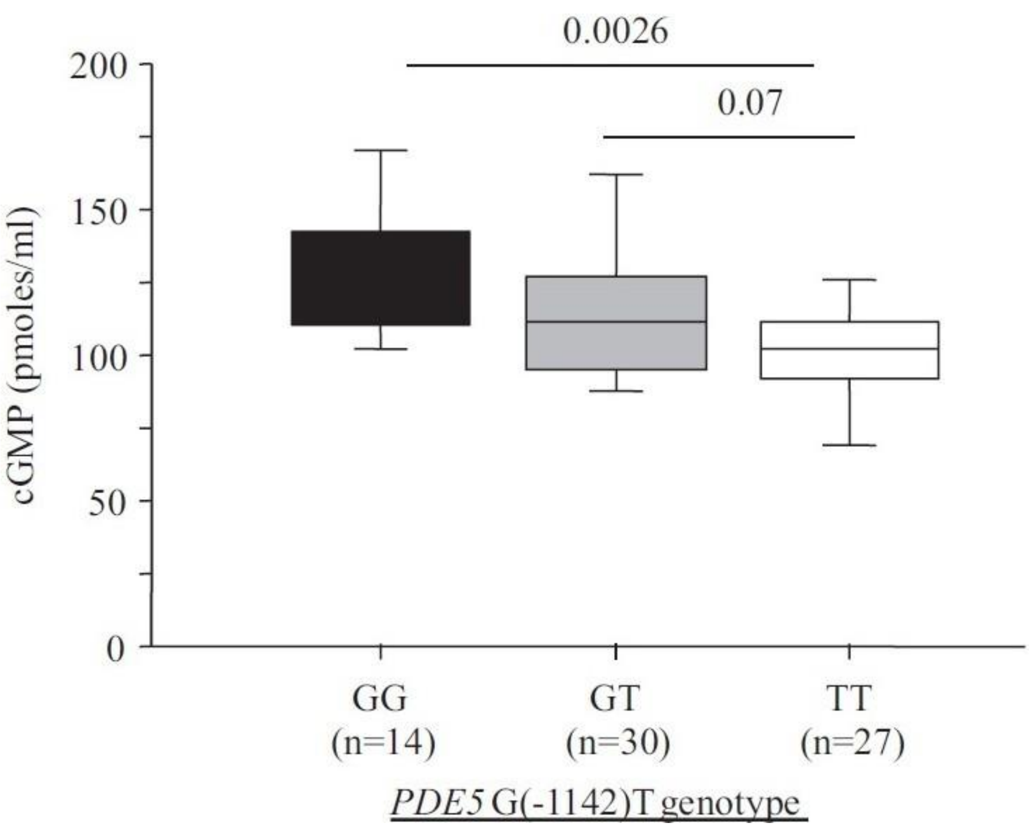
Case/control

- Output: OR and 95% confidence interval of the OR
- Test: is it significantly different from 1?
- Tests: Fisher's exact test or Chi-squared
- In case of dosages or covariates: logistic regression

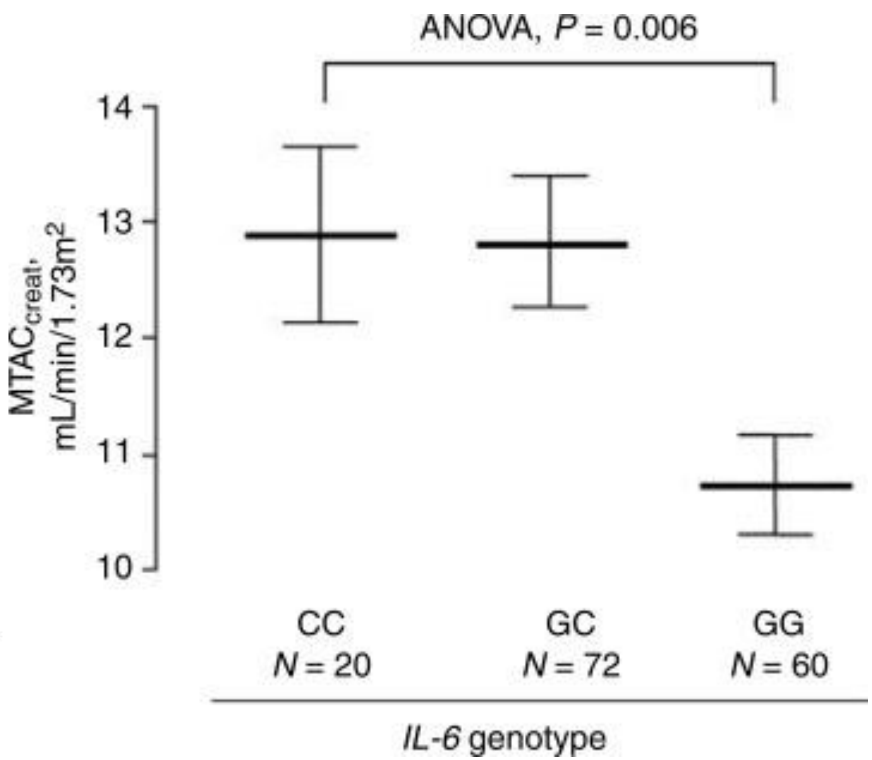
Data input

Continuous trait

- For directly typed (0,1,2): ANOVA



additive

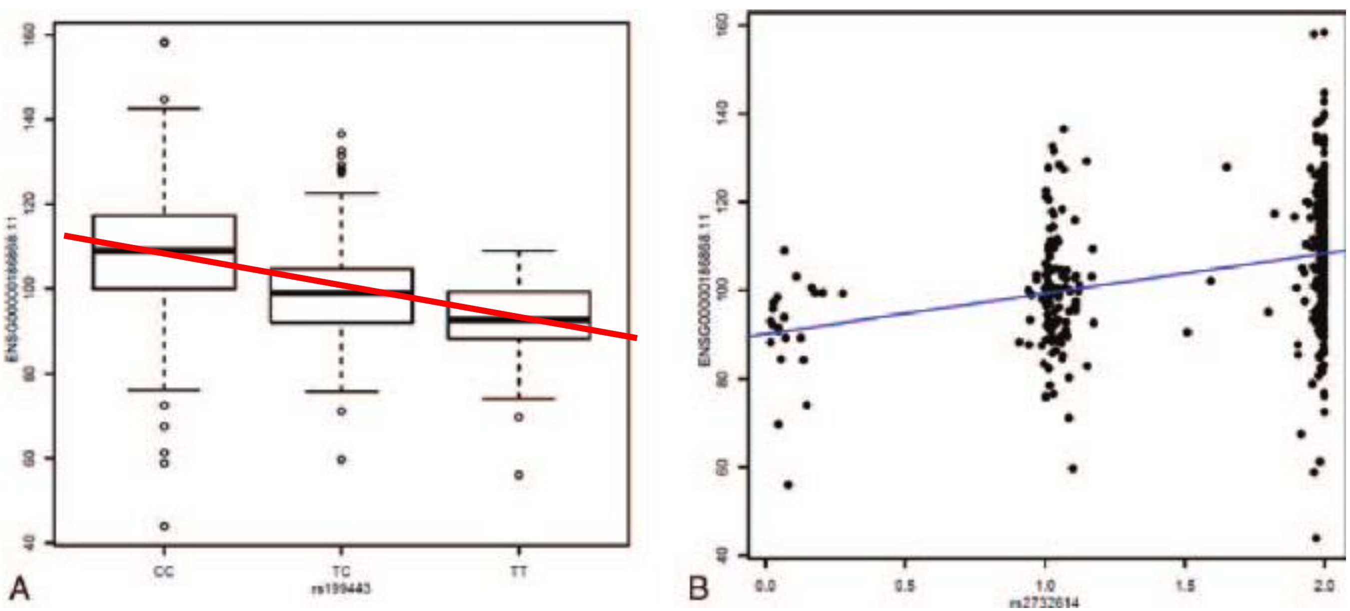


recessive

Data input

Continuous trait

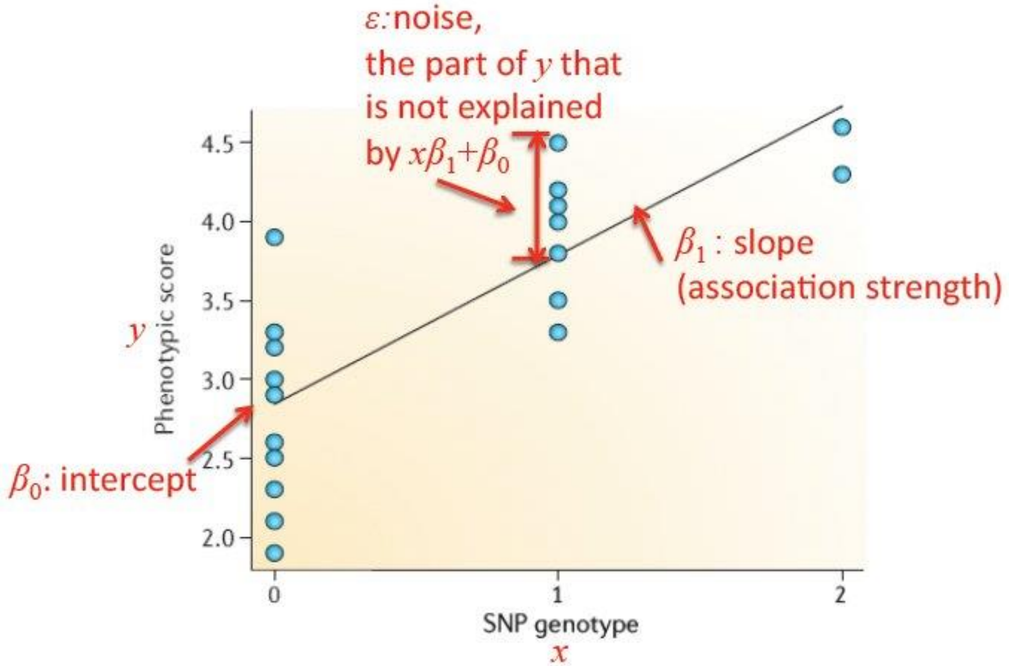
- For dosages (imputed quantity of minor allele $d \in [0,1]$) : linear regression
- In general: generalized linear model



Continuous trait

A linear regression model is defined as

$$y = x\beta_1 + \beta_0 + \varepsilon$$



Data:

- y: a continuous trait
- x: SNP genotype at a given locus

Parameters:

- β_1: regression coefficient, represents the strength of association between x and y
- β_o: intercept term (is 0 or ignored)
- ε: noise or the part of y that is not explained by x (e.g., environmental effect)

Assumptions:

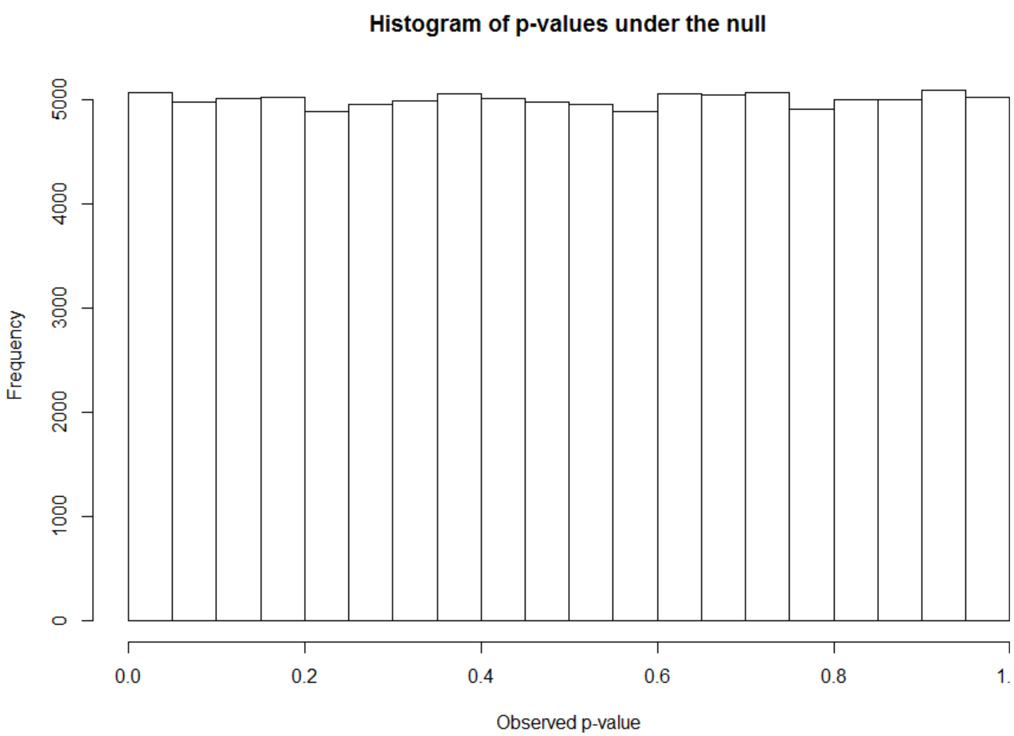
- The individuals in the study are not related
- The phenotype y has a normal distribution

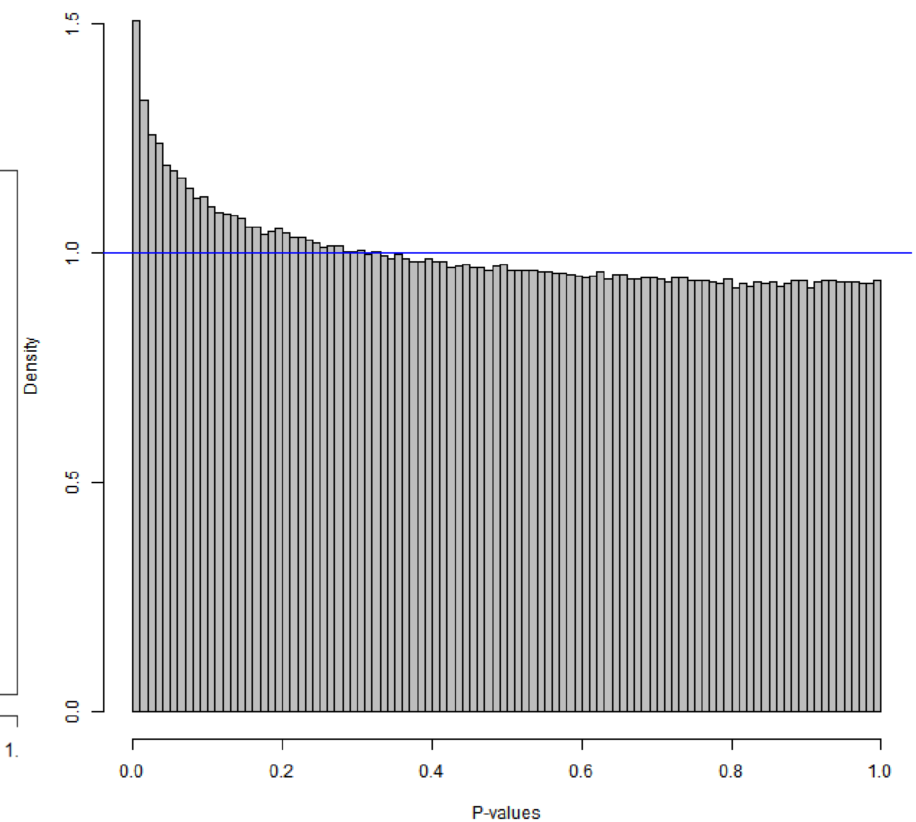
Checking results

- Statistical significance:
  - One test: $p<0.05$
  - Genome-wide: one test per variant and per phenotype
  - But all variants are not independent, in reality, we account for LD
  - 5x10-8 for GWAS, 10-9 for sequencing-based

Checking results

- QQ-plot
  - Distribution of p-values is uniform [0,1] under the null
  - If we have much signal, more around 0
  - Compare quantiles with expected ones : QQ-plot
  - In R: `qqunif`





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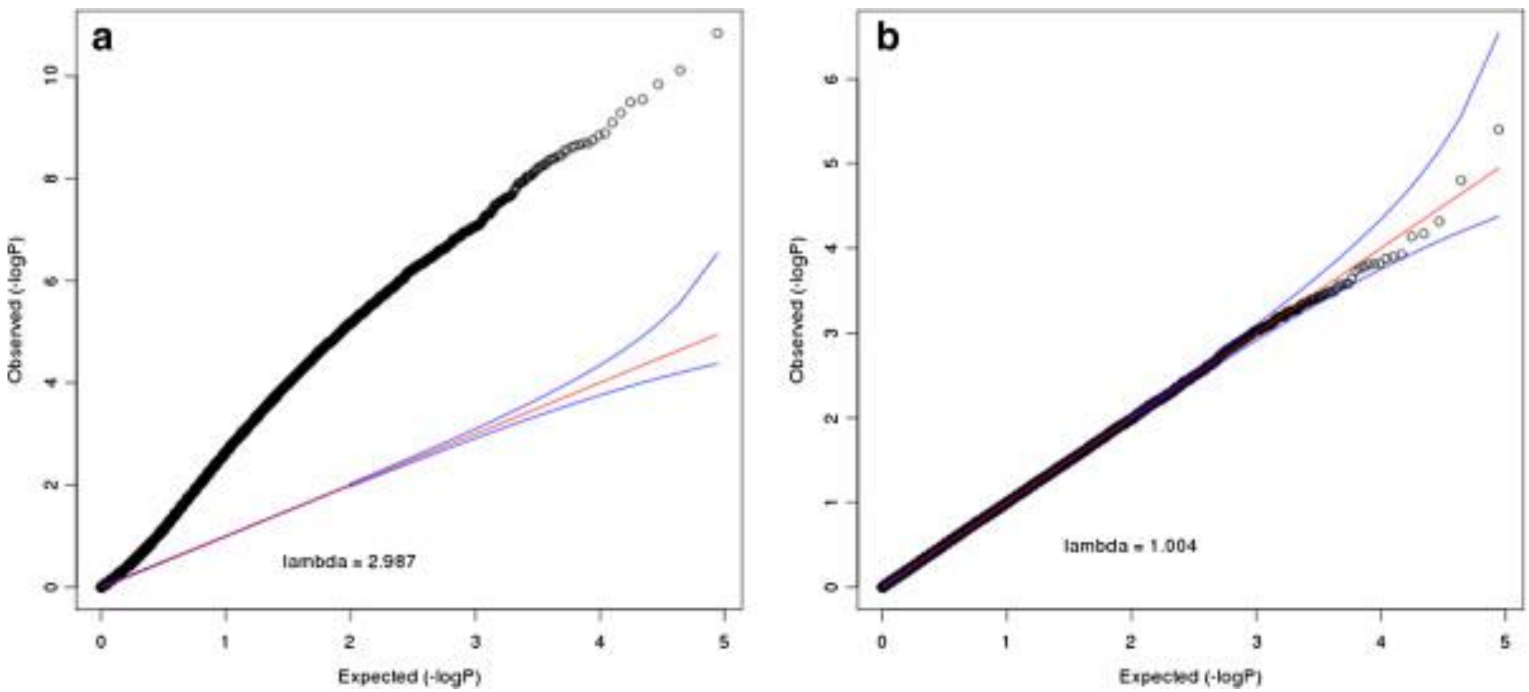

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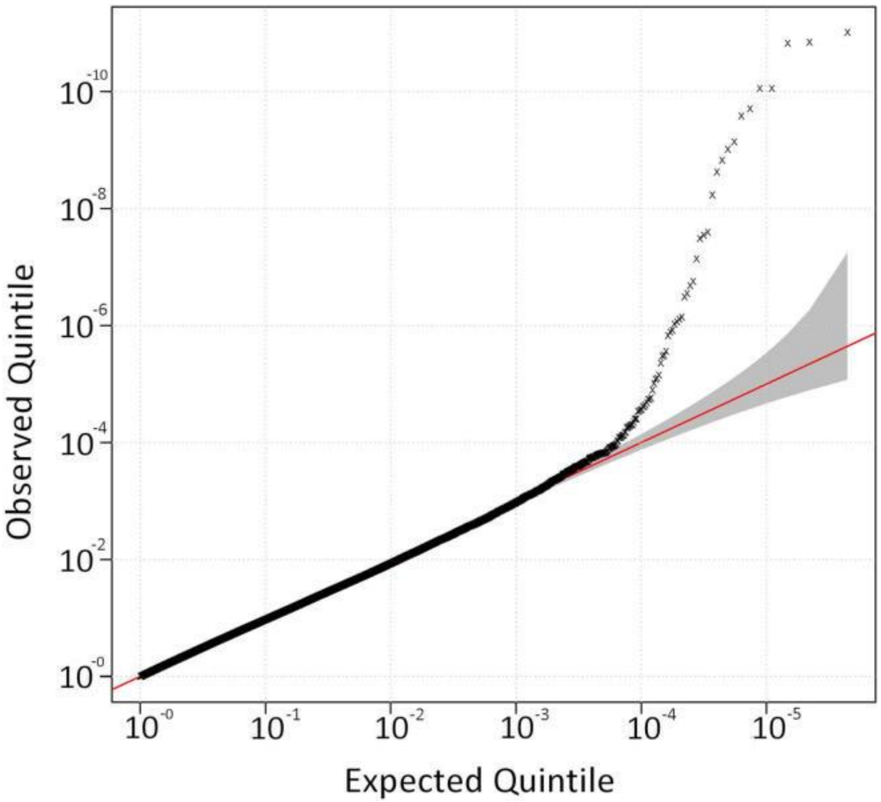

- Inflation: too much signal
- Measured visually, but also lambda

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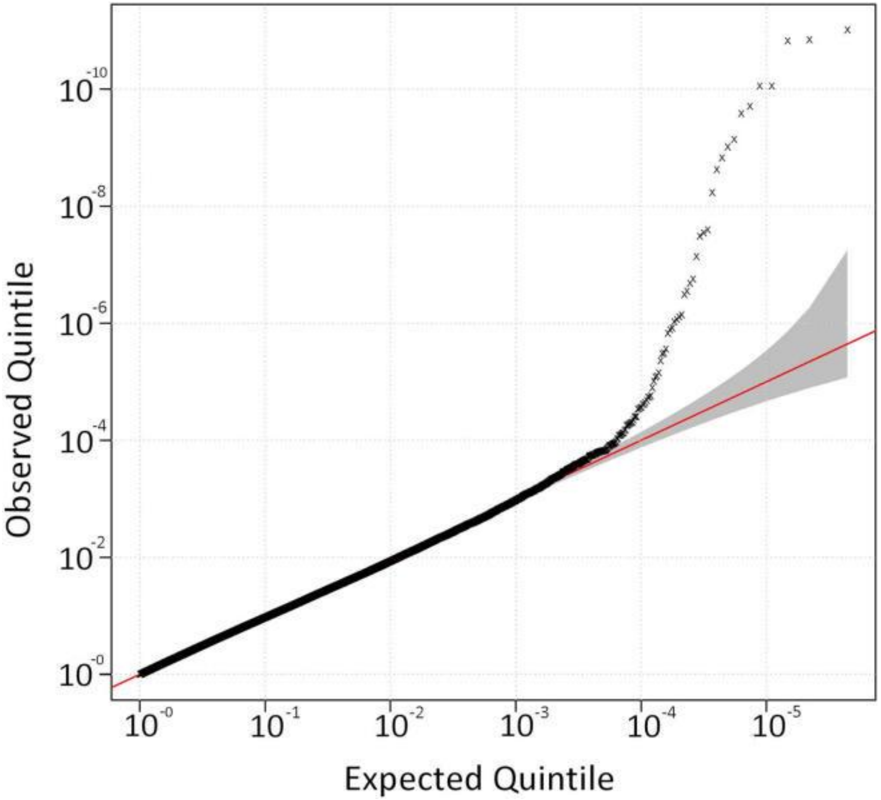

Appearances can be deceiving:

- A QQ-plot can look inflated when it isn't (just a lot of signal)
- And conversely
- We calculate the genomic inflation factor

$$\lambda = \frac{median(Q_{\chi^2}(p))}{0.45}$$

(median of χ^2 test statistics divided by median of χ^2_1)

Data input

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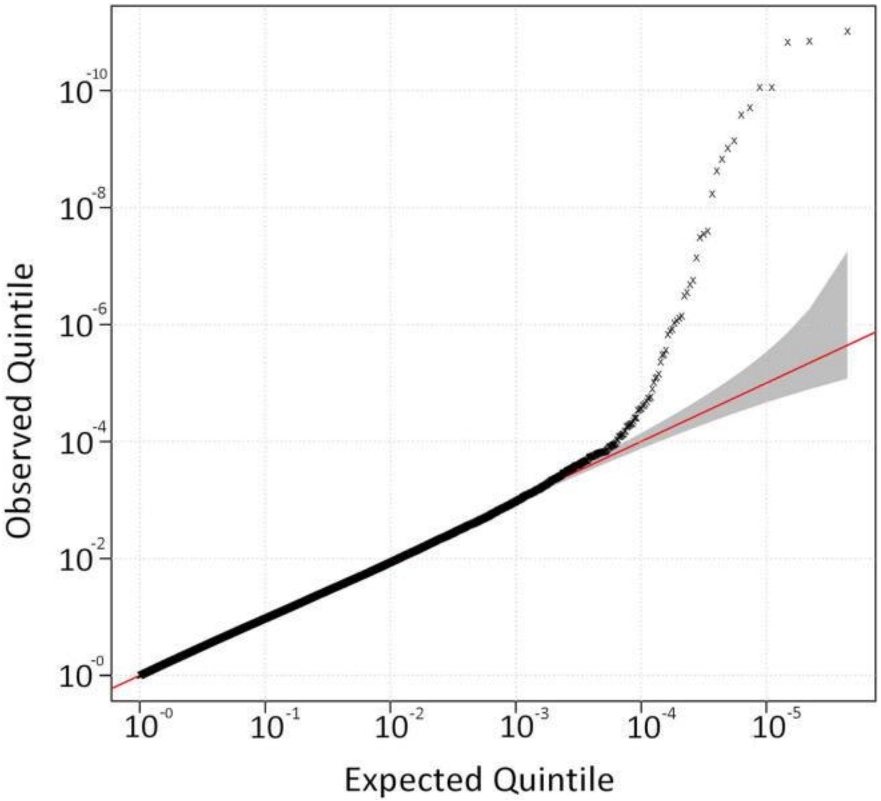

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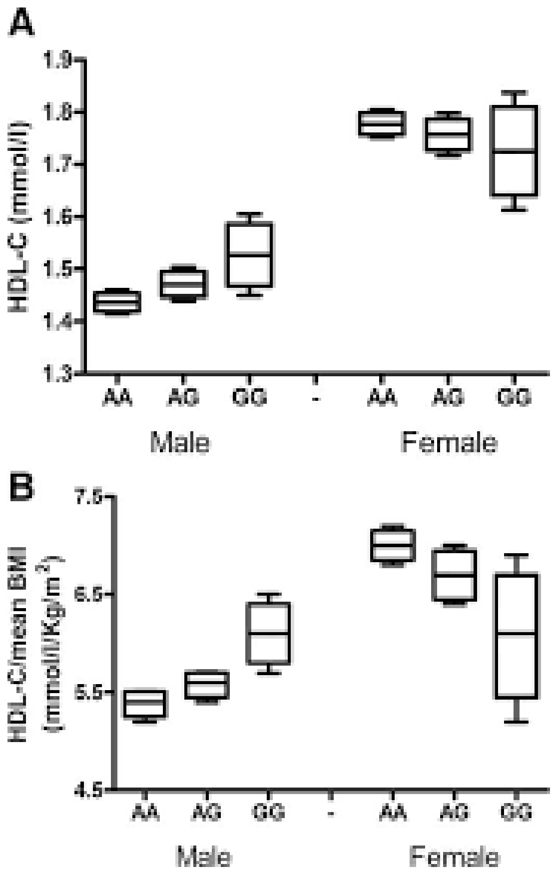
- Ideally, want to correct in the model
- Can also adjust: GC correction (divide by lambda)

Data input

Problems

- Covariates: sex, batch effects, chip effects



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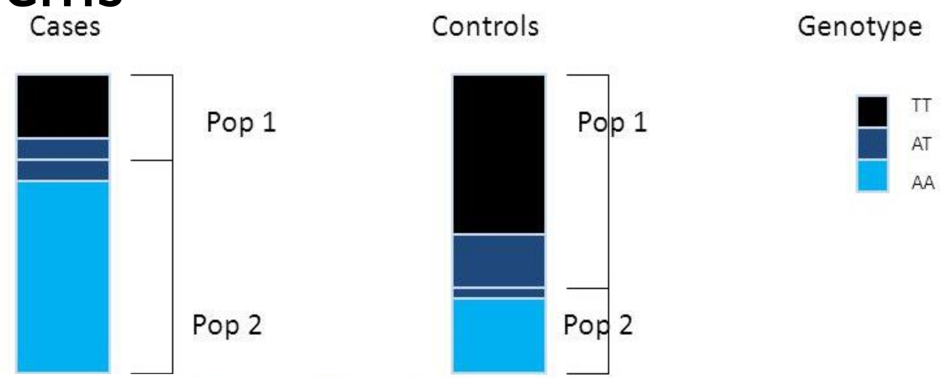
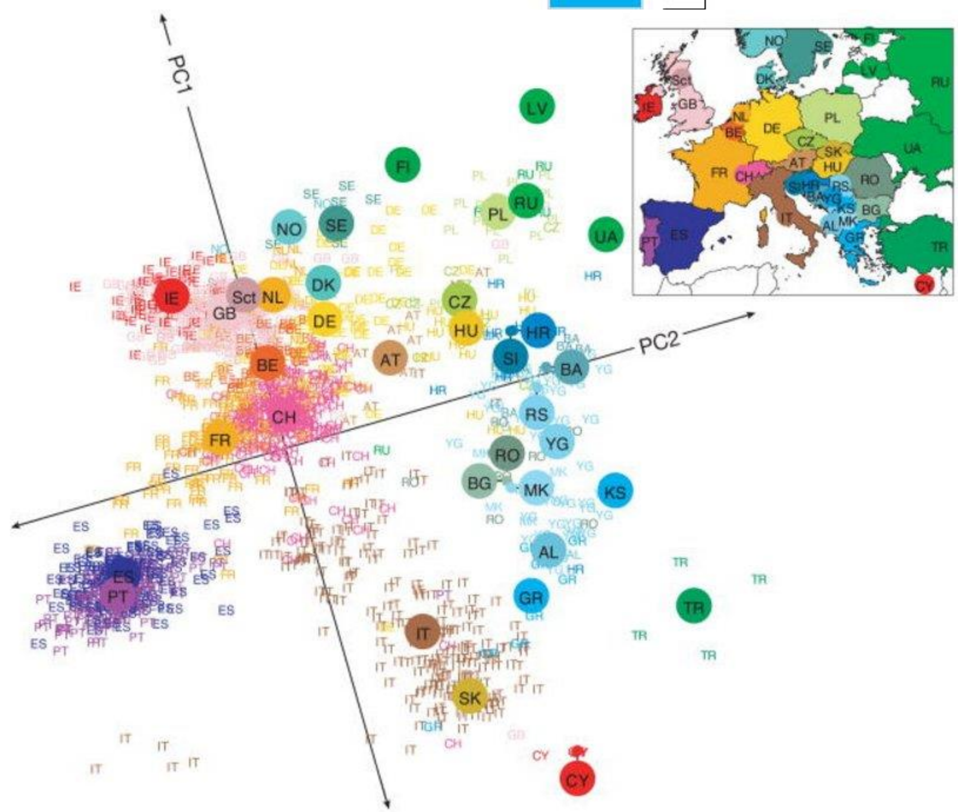
- Covariates: sex, batch effects, chip effects
- Structure: villages or subpopulations: linear mixed models can model the intra-group effect





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- Structure: villages or subpopulations: linear mixed models can model the intra-group effect

$$phenotype \sim \beta \times genotype + \beta_1 \times covariates + \beta_2 \times structure + \epsilon$$