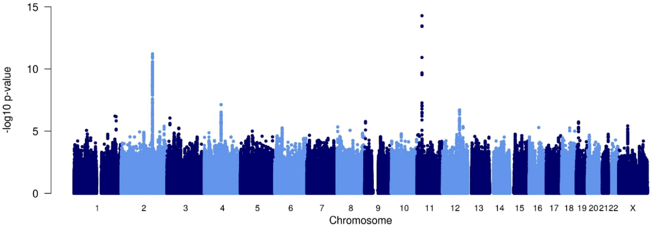
Association testing and GWAS



Line Skotte, Medical and Population Genetics Course, August 2018

Outline

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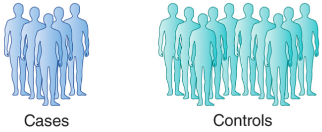
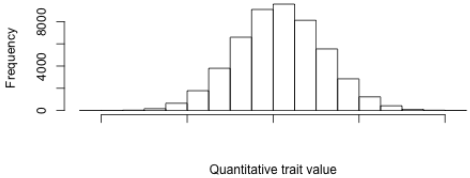
- Note, **can also be used in e.g. evolutionary studies!**

Plan for today (to teach you how)

- **This afternoon:**
  - How to test if a genetic variant potentially affects a trait (single SNP tests)
  - How to search the genome for variants that affect a given trait (GWAS)
  - We will assume we have genotyping data (e.g. from SNP chip)
  - We will assume there is no population structure
  - We will look at disease status traits:



  - And quantitative traits:



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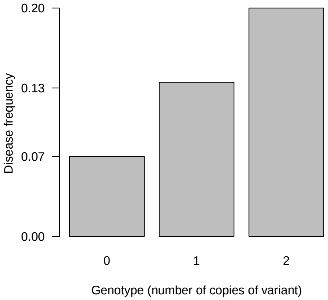

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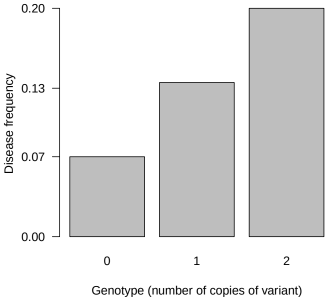

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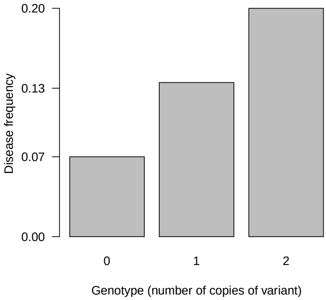

- Rationale: this is what we expect if the variant affects the trait
- Approach: test null hypothesis, H_0, of no association (independence)

χ^2 test for independence

- A test which you can apply to counts tables for two categorical variables
  E.g. disease status and genotypes:

	AA	Aa	aa	Total
Case	441	418	141	1000
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- We use this to decide whether we reject the null hypothesis
  (we reject when p is small and see it as evidence for association)

χ^2 tests - test with genotype counts

- Can be applied to genotype count tables
- So assume we have **observed** this data:

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Case	O_1=441	O_3=418	O_5=141	1000
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- **Small exercise:** what would E_3 and E_4 be?

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- Tells us that the probability of getting a X^2 value 16.5838 or higher **if** there is no association is low ($p \simeq 0.00025 < 0.05$)
- We therefore reject the null hypothesis of no association and conclude that the variant is associated with the disease status

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Counts of homozygous carriers vs others	AA or Aa	aa	Total
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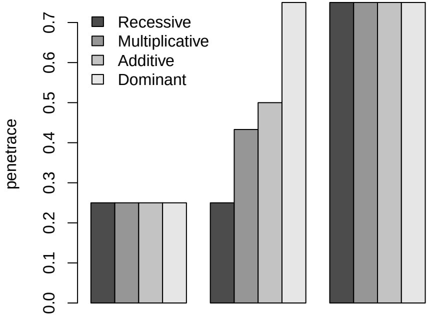
- How would you test assuming a dominant model?

Other inheritance models

- Commonly considered genetic inheritance models:



- Testing under an additive genetic inheritance models is more tricky can be done using e.g. an Armitage trend test
- Testing under a multiplicative model can be done using logistic regression

Logistic regression

- Based on the following general model

$$\log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_1^i + \ldots + \beta_n x_n^i$$

where the βs are *regression coefficients* (effect sizes).

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- Test if β_1 is zero (no association between the variant and the trait)

Why is logistic regression a good framework to use?

Logistic regression is very convenient due to its flexibility:

- Most inheritance models can be tested (by recoding x^i):

Genotypes	multiplicative	dominant	recessive	genotypes	
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- Can incorporate other factors in the model
  - discrete factors such as gender
  - continuous factors such as age

  Can be used to correct for possible confounding factors
  Can be used for metaanalysis by incl a factor for the different studies

Exercise

Let's try to perform some of these tests in R:

Solve exercise 1A, 1B, 1C and 1D (+ 1E if you have time)

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- This means that we see association in loci that are in high LD with the causal SNP
  So you have to be careful what you conclude from an association signal!

Other important limitations

One also has to be aware of the underlying assumptions:

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- If these assumptions are violated you risk getting false positives!
- Hence Quality Control (QC) and appropriate modelling is crucial!

Effect sizes for case-control data - relative risk

Relative risk - definition

$$RR = \frac{P(Case|Exposed)}{P(Case|Not\ exposed)}$$

where exposed depends on model, e.g. exposed=aa under recessive model

I.e. how many times higher the *risk* of disease is for exposed

Relative risk - example with recessive model

	Cases	Controls	Total
Exposed (g=aa)	100	100	200
Not exposed (g=AA or Aa)	400	3600	4000

- $P(Case|Exposed) = \frac{100}{200} = \frac{1}{2}$
- $P(Case|Not\ exposed) = \frac{400}{4000} = \frac{1}{10}$
- $RR = \frac{1/2}{1/10} = 5$

Effect sizes for case-control data - odds ratio

Odds ratio - definition

$$OR = \frac{ODD_{Exposed}}{ODD_{Not\ \ Exposed}} = \frac{\frac{P(Case|Exposed)}{P(Control|Exposed)}}{\frac{P(Case|Not\ \ exposed)}{P(Control|Not\ \ exposed)}}$$

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Odds ratio - example with recessive model

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Not exposed (g=AA or Aa)	400	3600	4000

- $\frac{P(Case|Exposed)}{P(Control|Exposed)} = \frac{100/200}{100/200} = \frac{100}{100} = 1$
- $\frac{P(Case|Not\ \ exposed)}{P(Control|Not\ \ exposed)} = \frac{400/4000}{3600/4000} = \frac{400}{3600} = 1/9$
- $OR = \frac{1}{1/9} = 9$ (very high for an association study!)

Effect size estimates from logistic regression

- In logistic regression the ORs are estimated directly:
  In the model we estimate the effect size β_1

$$\log\left(\frac{p_i}{1-p_i}\right) = \beta_0 + \beta_1 x_1^i \ldots$$

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- If time allows do exercise 1F

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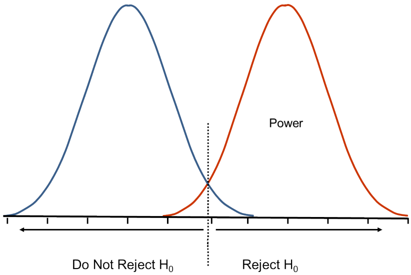

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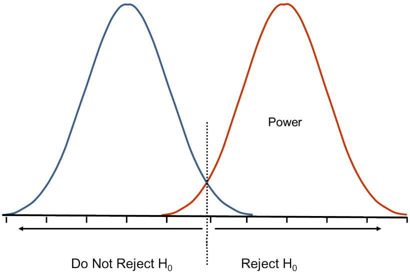

Crucial for whether the study is worth performing!

- Before you start your study: calculate power for your study and assess it
  Rule of thumb: power should be at least 0.8

Power and power calculations

- Power depends on
  - the inheritance mode, e.g. recessive effect
  - the effect size, e.g. OR of 1.3 (the bigger the higher power)
  - the frequency of allele, e.g. 0.04 (the bigger the higher power)
  - **the rejection criterion**, e.g. $p < 0.05$ (the bigger the higher power)
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  Do power calculations to make sure you will have enough samples!

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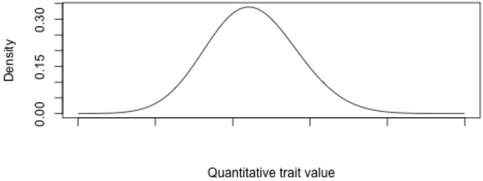
- Can often be calculated using "power-calculators"
- So before you start:
  Do power calculations to make sure you will have enough samples!
- To detect association we might not choose the model that is most correct, but instead choose the model that has the most power

Outline

Quantitative trait

- Distribution of the trait in the population



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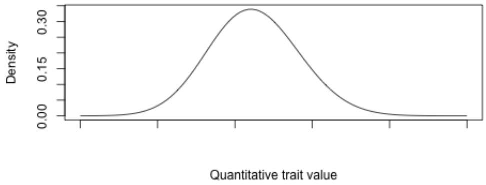
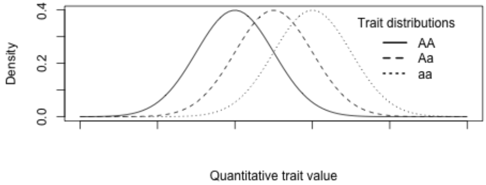


- If a variant influence the trait value, we expect:



Linear regression

- Based on the following general model

$$E(y_i) = \beta_0 + \beta_1 x_1^i + \ldots + \beta_n x_n^i$$

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- Test if β_1 is zero (no association between the variant and the trait)

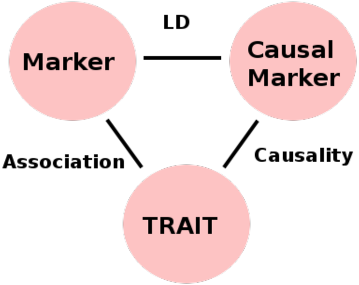
Outline

Types of association studies

- Candidate causative genetic variant
  - 1 SNP or deletion, duplication. Evidence from other study
- Candidate causative gene
  - 5-50 SNPs. Evidence from other study or function
- Candidate causative region
  - 100s of SNPs Evidence from other study
- Genome-wide (GWAS)
  - $>$500,000 SNPs. No prior evidence required

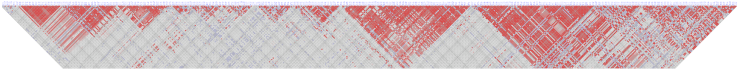
Why GWAS?

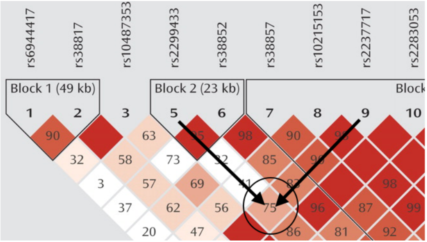
- If we look at 500.000 SNPs we are likely not to have the causal SNP!
- But, remember SNPs in high LD with a causal SNP will also be associated:



Why GWAS?

- SNPs are in high LD in blocks along the human genome





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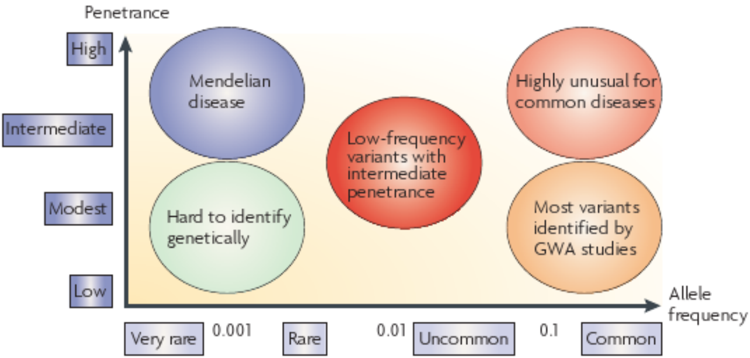
- We can test most common SNPs (indirectly) by using $\geq 500,000$ SNPs
- Pro: Cheap! (only need to genotype $\geq 500,000$ SNPs)
  Con: We are far from sure the identified SNPs (if any) are causal!

When GWAS?



Strategies for locating disease loci

How GWAS (step-by-step overview)

1. Collect samples and traits of interest (based on power calculations!)
2. Genotype samples at a number of SNP loci ($\geq$500,000)
3. Lots and lots of quality control (QC)!
4. Statistically test each SNP for association
5. Assess the results:
    - make sure things went OK
    - identify associated SNPs
6. Identify causal variant (if possible)
7. Replicate associations in a different dataset
8. Investigate what the underlying biological mechanism is
9. Ideal longterm goal/hope: better prevention or treatment

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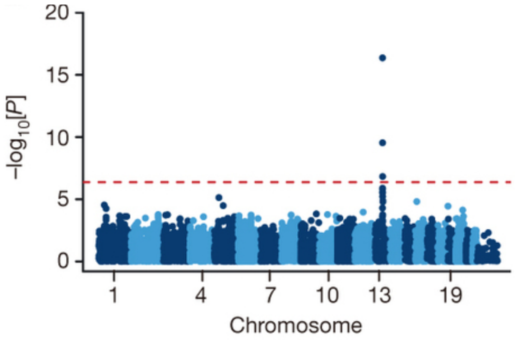
Statistically test each SNP for association

- Use one of the tests you just learned how to perform
- There are programs like PLINK2 that will help you do this
- Can be done using one 1-line command
- Also offers functions for doing QC (we'll see that later)

Identify associated SNPs

Manhattan plot



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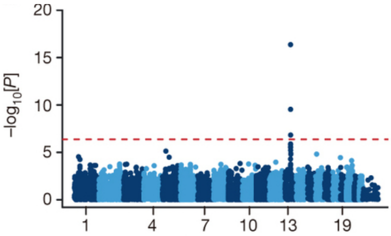

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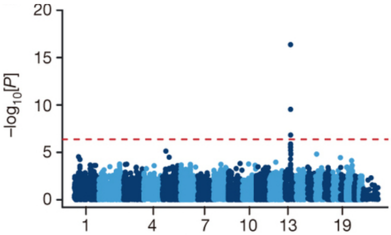
  

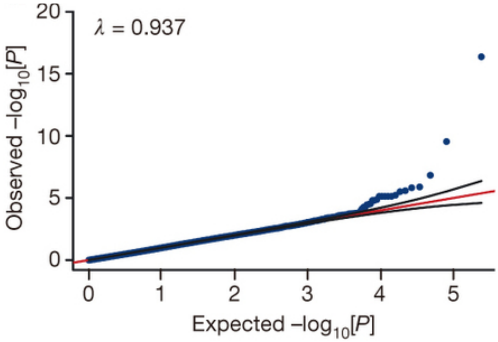
  So we have to **correct for multiple testing**
- Often **Bonferroni correction** is used; α is divided by the number of tests:
  - E.g. 100000 SNPs and $\alpha = 0.05$
  - Bonferroni corrected $\alpha = 0.05/100000 = 0.0000005 = 5 \times 10^{-7}$
  - Which on the Manhattan plot is $-log_{10}(5 \times 10^{-7}) = 6.3$

Exercise

Solve exercise 2A, i.e. perform your first GWAS analysis :)

Make sure things went OK!

QQ-plots and genomic control inflation factor λ



If so most of the dots will be on the x=y line and $\lambda \simeq 1$

Exercise

Solve exercise 2B, i.e. check if your results look OK...

Lots and lots of QC

This shows why we usually do QC first ...! :)

Let's therefore return to that step
(we wont go through all QCs, but some important ones)

Sample mislabling?

- One thing that can go wrong is the samples can be misslabled
- If so, genotypes won't match phenotypes
- This is difficult to catch
- But a simple check is to see of gender is correct
- If not the disease status is likely not to be either...
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- **Exercise:** try checking it for your data (exercise 2C)

Closely related individuals or duplicates?

- All association tests mentioned assume that the participants are **independent** samples from a population
- This would not be the case if some participants
  - are closely related
  - represented more than once
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Batch biases/non-random genotyping error?

- Sometimes the data handling/generation process can lead to non-random genotyping errors
- E.g. if all cases were genotyped first and then all controls, then changes in genotyping procedure along the way may lead to non-random differences in genotypes between cases and controls
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- This may lead the false positive association test results
- **Exercise:** try checking it for your data (exercise 2E+F if there is time)

Additional important checks?

- Other additional signs of something being wrong include:
  - high missingness in specific loci/individuals
  - loci (strongly) out of Hardy-Weinberg Equilibrium (why?)
- Furthermore, low frequency variants tend to be difficult to genotype
- Removing such loci/individuals can help a lot

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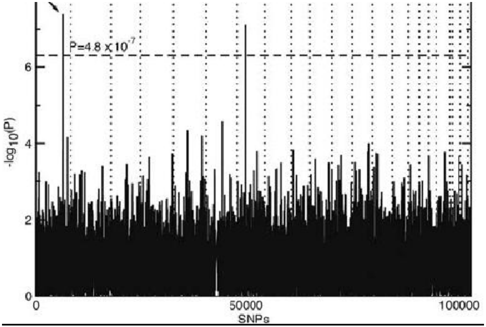
- Furthermore, low frequency variants tend to be difficult to genotype
- Removing such loci/individuals can help a lot
- **Exercise:** try rerunning your analyses with these QC filters (exercise 2G)

First study went extremely well!

- Study of age-related Macular Degeneration (Klein et al. 2005)
- 96 cases and 50 controls, 100K SNPs



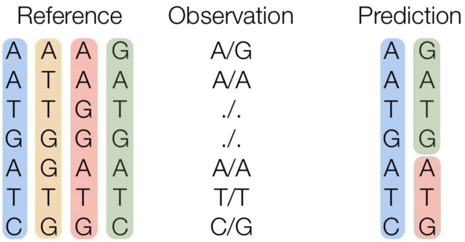
- SNP in *CFH* w large effect (OR=7.4)+led to new biological insight

Turned out to be unusual...

- MANY studies and many associations
- But in the beginning few were replicated
  (underpowered, population structure, insufficient corr. for multiple tests)
- So later studies have many more samples and are much stricter
- And most found small effect sizes and limited biological insight

NGS enters the stage

- Reference panels
  - 1000 genomes project
  - Haplotype reference consortium
- Imputation:



- Results in posterior genotype probabilities.

Dealing with uncertain genotypes in associations

- The easy solution: DOSAGE

$$E[g] = \sum_{g=0}^{2} g \; p(G = g|X)$$

- The complicated solution: Full likelihood model

$$p(y|X) = \prod_i \sum_g p(y_i|G_i = g)p(G_i = g|X_i)$$

- Same goes for association studies based on directly on sequencing data.