Lecture 1: Case Control Association Testing & Association Testing with Quantitative Traits

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Summer Institute in Statistical Genetics 2022

Introduction

- Association mapping is now routinely being used to identify loci that are involved with complex traits.
- Technological advances have made it feasible to perform association studies on a genome-wide basis with millions of markers in a single study.
- We consider testing a genetic marker for association with a disease (e.g. 1/0, affected/unaffected, dead/alive) or a quantitative trait (e.g. height, BMI) in **a sample of unrelated subjects**.
- Vast amounts of literature on these topics!

Case-Control Association Testing

- Allelic Association Tests
  - Allele is treated as the sampling unit
  - Typically make an assumption of Hardy-Weinberg equilibrium (HWE) - alleles within an individual are conditionally independent given the disease status
  - e.g. Pearson's χ^2
- Genotypic Association Tests
  - Individual is the sampling unit
  - Does not assume HWE
  - e.g. Logistic regression

Pearson's χ^2 Test for Allelic Association

- This test looks for deviations from independence between the trait and allele.
- Consider a single marker with 2 allelic types (e.g., a SNP) labeled "C"' and "T"'.
- Let N_{ca} be the number of cases and N_{co} be the number of controls on which we have genotype data.

Pearson's χ^2 Test for Allelic Association

- Below is a 2×2 contingency table for trait and allelic type

	Cases	Controls	Total
Allele C	n_C^{ca}	n_C^{co}	N_C
Allele T	n_T^{ca}	n_T^{co}	N_T
Total	$2N_{ca}$	$2N_{co}$	$2N$

- n_C^{ca} is the number of C alleles in the cases and $n_C^{ca} = 2 \times$ the number of homozygous CC cases $+$ the number of heterozygous CT cases
- Hypotheses
  - H_0: there is *no association* between the row variable and column variable
  - H_a: there *is* an association between the two variables

Pearson's χ^2 Test for Allelic Association

- Can use Pearson's χ^2 test for independence. The statistic is:

$$X^2 = \sum_{\text{all cells}} \frac{(\text{Observed cell} - \text{ Expected cell})^2}{\text{Expected cell}}$$

- What is the the expected cell number under H_0? For each cell, we have

$$\text{Expected Cell Count} = \frac{\text{row total } \times \text{ col total}}{\text{total count}}$$

- Under H_0, the X^2 test statistic has an approximate χ^2 distribution with $(r-1)(c-1) = (2-1)(2-1) = 1$ degree of freedom

LHON Example: Pearson's χ^2 Test

- From Phasukijwattana et al. (2010), Leber Hereditary Optic Neuropathy (LHON) disease and genotypes for marker rs6767450:

	CC	CT	TT
Cases	6	8	75
Controls	10	66	163

- Corresponding 2×2 contingency table with allelic type instead of genotype

	Allele C	Allele T
Cases	20	158
Controls	86	39

- Should we reject the hypothesis that allelic type is independent of disease status?

LHON Example: Pearson's χ^2 Test

	Allele C	Allele T	Total
Cases	20	158	178
Controls	86	392	478
Total	106	550	656

- Intuition for the test: Suppose H_0 is true, so allelic type and case-control status are independent, then what counts would we expect?
  - the expected number of case alleles that are of type C is:

$$\begin{aligned} n^{ca}_{T} &= \{\#cases\} \times P(\text{Allelic type is C} \mid \text{Allele is from a Case}) \\ &= \{\#cases\} \times P(\text{Allelic type is C}) \quad \textbf{(independence)} \\ &= 178 \times \left(\frac{106}{656}\right) = 28.7622 \end{aligned}$$

LHON Example: Pearson's χ^2 Test

- Fill in the remaining cells for the expected counts

	Allele C	Allele T	Total
Cases	28.7622	.	178
Controls	.	.	478
Total	106	550	656

LHON Example: Pearson's χ^2 Test

- Fill in the remaining cells for the expected counts

	Allele C	Allele T	Total
Cases	28.7622	149.2378	178
Controls	77.2378	400.7622	478
Total	106	550	656

LHON Example: Pearson's χ^2 Test

- Fill in the remaining cells for the expected counts

	Allele C	Allele T	Total
Cases	28.7622	149.2378	178
Controls	77.2378	400.7622	478
Total	106	550	656

- Calculate the X^2 statistic

$$X^2 = \frac{(20 - 28.7622)^2}{28.7622} + \cdots + \frac{(392 - 400.7622)^2}{400.7622} = 4.369$$

LHON Example: Pearson's χ^2 Test

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- Calculate the X^2 statistic

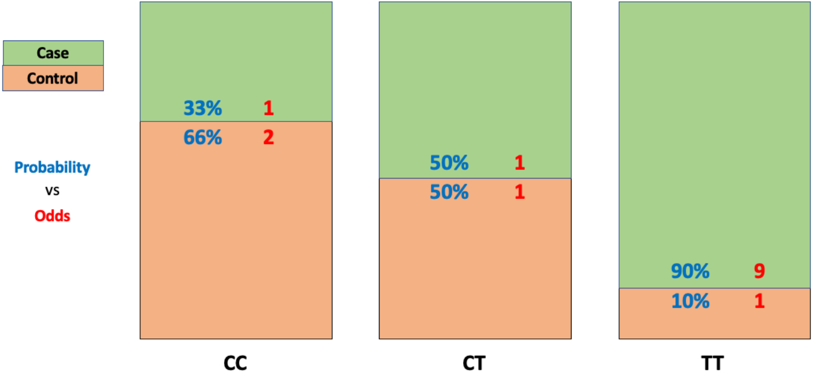
$$X^2 = \frac{(20 - 28.7622)^2}{28.7622} + \cdots + \frac{(392 - 400.7622)^2}{400.7622} = 4.369$$

- What is the p-value?

$$P(\chi^2_1 \geq 4.369) = .037$$

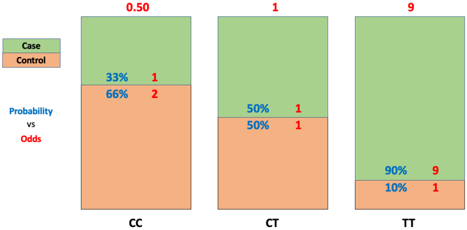
Odds Ratios (ORs) for Genotypes

- What are **odds**? An expression of **relative probabilities**...



- Odds of disease in an individual with the CC genotype = 50%

Odds Ratios (ORs) for Genotypes



- Typically choose a reference genotype, e.g. CC

$$OR_{TT} = \frac{\text{odds of disease with the TT genotype}}{\text{odds of disease with the CC genotype}} = \frac{9}{0.50} = 18$$

- $OR_{TT} = 1$ implies no association with disease.
- $OR_{TT} > 1$ or $OR_{TT} < 1$ implies association with the disease.

Genotypic Association Tests: Logistic regression

- Generally used to estimate odds ratios and get confidence intervals for genotypes.
- Let π_i be the probability that individual i is has the disease and let G_i be the genotype at the SNP:

$$\underbrace{\log\left(\frac{\pi_i}{1-\pi_i}\middle| G_i\right)}_{\text{odds of disease}} = \beta_0 + \beta_{CT} I\{G_i = CT\} + \beta_{TT} I\{G_i = TT\}$$

where $I\{G_i = CT\}$ is 1 if $G_i = CT$ and 0 otherwise, and similarly for $I\{G_i = TT\}$.

Genotypic Association Tests: Logistic regression

- The coefficient estimates for $\hat{\beta}_{CT}$ and $\hat{\beta}_{TT}$ can be used to calculate odds ratios:

$$\begin{aligned} OR_{CT} &= \frac{\text{odds of disease with the CT genotype}}{\text{odds of disease with the CC genotype}} \\ &= \frac{exp(\hat{\beta}_0 + \hat{\beta}_{CT})}{exp(\hat{\beta}_0)} = exp(\hat{\beta}_{CT}) \end{aligned}$$

  Similarly, $OR_{TT} = exp(\hat{\beta}_{TT})$

- 95% CI for OR_{CT} is

$$exp(\hat{\beta}_{CT} \pm 1.96 \times s.e.(\hat{\beta}_{CT}))$$

Odds Ratios for LHON Example

- Leber Hereditary Optic Neuropathy (LHON) disease and genotypes for marker rs6767450:

	CC	CT	TT
Cases	6	8	75
Controls	10	66	163

- We will use the R to obtain odds ratios and confidence intervals for this data set
- We will contrast this test with an allelic test (Pearson's χ^2 test).

Introduction to Quantitative Trait Mapping

- Quantitative trait loci (QTL) mapping involves identifying genetic loci that influence the phenotypic variation of a quantitative trait.
- QTL mapping is commonly conducted with GWAS, often involving directly genotyped variants along with imputation through reference panels to result in millions of genetic variants
- Some quantitative traits can be largely influenced by a single gene as well as by environmental factors (e.g. monogenic or Mendelian traits)

Introduction to Quantitative Trait Mapping

- Influences on a quantitative trait can also be due to a number of genes (polygenicity)
- Many quantitative traits of interest are complex where phenotypic variation is due to a combination of both multiple genes and environmental factors
- Examples: Blood pressure, cholesterol levels, IQ, height, weight, etc.

Quantitative Genetic Model

- The classical quantitative genetics model introduced by Ronald Fisher (1918) is $Y = G + E$, where Y is the phenotypic value, G is the genetic value, and E is the environmental deviation.
- G is the combination of all genetic loci that influence the phenotypic value and E consists of all non-genetic factors that influence the phenotype
- The mean environmental deviation E is generally taken to be 0 so that the mean genotypic value is equal to the mean phenotypic value, i.e., $E(Y) = E(G)$

Components of Genetic Variance

- Consider a single locus. Fisher modeled the genotypic value G with a linear regression model (least squares) where the genotypic value can be partitioned into an additive component (A) and deviations from additivity as a result of dominance (D), where

$$G = A + D,$$
$$\underbrace{Var(G)}_{\sigma_G^2} = \underbrace{Var(A)}_{\sigma_A^2} + \underbrace{Var(D)}_{\sigma_D^2}$$

- σ_A^2 is the **additive genetic variance**. It is the genetic variance associated with the average additive effects of alleles
- σ_D^2 is the **dominance genetic variance**. It is the genetic variance associated with the dominance effects.

Heritability

- Remember that $Y = G + E = A + D + E$
- **Narrow-sense heritability** (or simply heritability) is defined as
$$h^2 = \frac{\sigma_A^2}{\sigma_Y^2}$$
- h^2 is the proportion of the total phenotypic variance that is due to additive effects.
- Heritability can also be viewed as the extent to which phenotypes are determined by the alleles transmitted from the parents.

Heritability

- The **broad-sense heritability** is defined to be

$$H^2 = \frac{\sigma_G^2}{\sigma_Y^2}$$

- H^2 is the proportion of the total phenotypic variance that is due to all genetic effects (additive and dominance)
- Heritability can vary over time and with the study population as it depends also on environmental effects

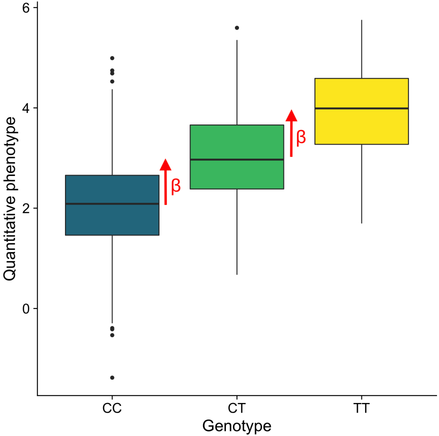
QTL Mapping

- For traits that are heritable, i.e., traits with a non-negligible genetic component that contributes to phenotypic variability, identifying (or mapping) QLT that influence the trait is often of interest.
- Linear regression models are commonly used for QTL mapping
  - They will often include a single genetic marker (e.g., a SNP) as predictor in the model, in addition to other relevant covariates (e.g. age, sex), with the quantitative phenotype as the response

Linear regression with SNPs

Many analyses fit the ‘additive model’

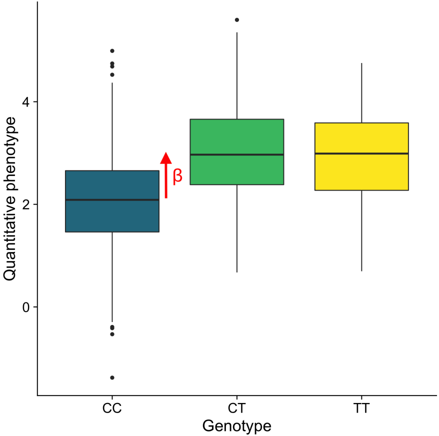
$$y = \beta_0 + \beta \times \#\text{T alleles}$$



Linear regression, with SNPs

An alternative is the 'dominant model';

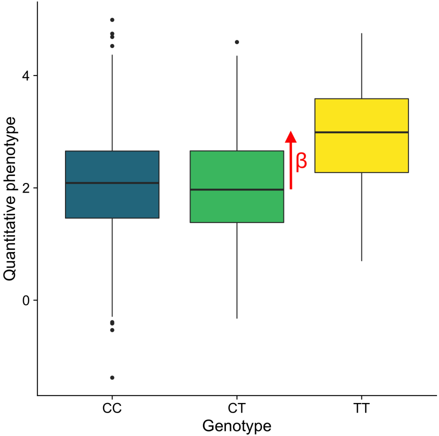
$$y = \beta_0 + \beta \times I\{G \neq CC\}$$



Linear regression, with SNPs

or the ‘recessive model’;

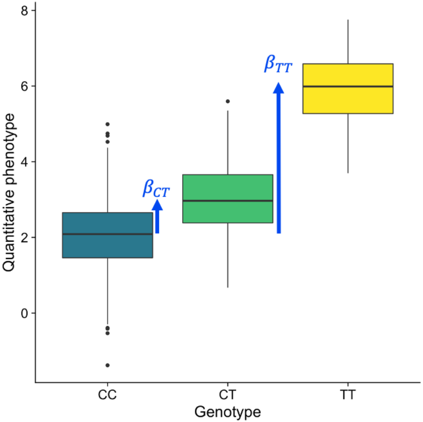
$$y = \beta_0 + \beta \times I\{G == TT\}$$



Linear regression, with SNPs

Finally, the ‘two degrees of freedom model’;

$$y = \beta_0 + \beta_{CT} \times I\{G == CT\} + \beta_{TT} \times I\{G == TT\}$$



Additive Genetic Model

- Most GWAS perform single SNP association testing with linear regression assuming an additive model.
- The coefficient of determination (r^2) of an additive linear regression model gives an estimate of the proportion of phenotypic variation that is explained by the SNP (or SNPs) in the model, e.g., the "SNP heritability"

Additive Genetic Model

- Consider the following additive model for association testing with a quantitative trait and a SNP with alleles C and T:

  $$Y = \beta_0 + \beta_1 G + \epsilon$$

  where G is the number of copies of the allele T.
- What would your interpretation of ϵ be for this particular model?

Association Testing with Additive Model

$$Y = \beta_0 + \beta_1 G + \epsilon$$

- Two test statistics for $H_0 : \beta_1 = 0$ versus $H_a : \beta_1 \neq 0$

$$T = \frac{\hat{\beta}_1}{\sqrt{var(\hat{\beta}_1)}} \sim \mathbf{t}_{N-2} \approx N(0,1) \text{ for large } N$$

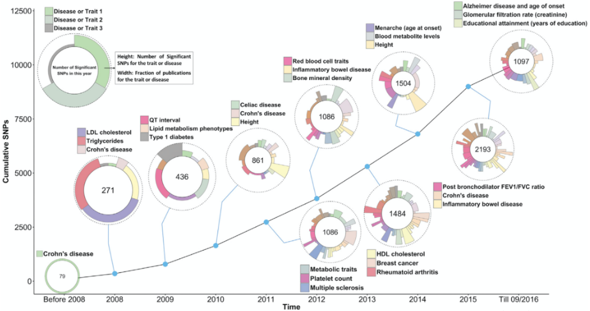
$$T^2 = \frac{\hat{\beta}_1^2}{var(\hat{\beta}_1)} \sim \mathbf{F}_{1,N-2} \approx \chi_1^2 \text{ for large } N$$

where

$$var(\hat{\beta}_1) = \frac{\sigma_\epsilon^2}{S_{GG}}$$

and S_{GG} is the corrected sum of squares for the G_i's

Missing Heritability



Visscher et al., *AJHG*, 2017

- Gap between SNP-based and pedigree-based heritability estimates
- Causal variants not well tagged? Rare variants involved?