



Centre for
Tropical Livestock
Genetics and Health

Introduction to GWAS

Ozzie Matika, Chrissy Rochus
& Isidore Houaga

Roslin Institute, University of Edinburgh

Slides adapted from Ivan Pocrić





Basics already covered

- **VERY BASIC Genetics ...!**
- **Genetic models for complex traits**
 - Heritabilities and variance partitioning
 - **SNP associations**
 - **Genomic selection**
- **What geneticists do ...**

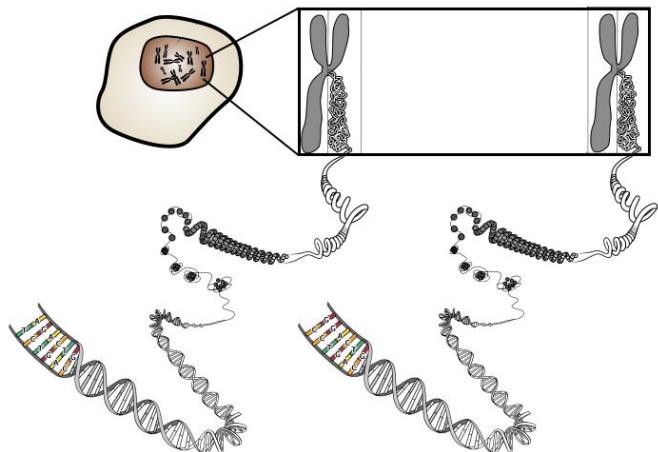


Refresher on genomic data

DNA, $\sim 3 \times 10^9$ base pairs x 2

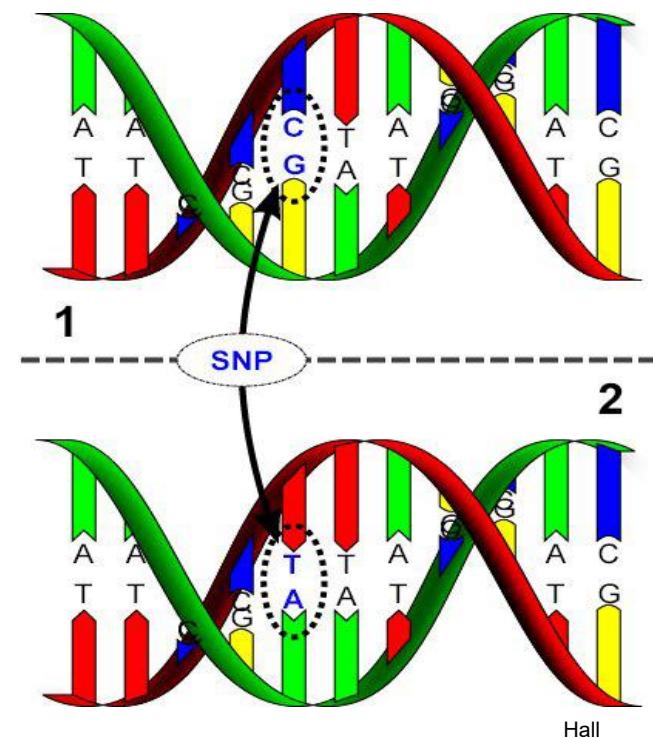
SNP array genotyping $\sim <100$ to 10^{5-6} variants

Sequencing $\sim 10^{7-8}$ variants



| Haplotypes |
|-------------------------|
| Id1 0 0 1 1 1 1 0 0 ... |
| Id1 1 0 1 1 1 0 0 0 ... |
| Id2 0 0 0 1 1 1 1 1 ... |
| Id2 0 0 0 1 1 0 0 0 ... |
| ... |

| Genotypes |
|---------------------------|
| Id1 1 0 2 2 2 2 1 0 0 ... |
| Id2 0 0 0 2 2 1 1 1 ... |
| ... |





Refresher on genomic data

The sequences of >150,119 genomes in the UK biobank

<https://doi.org/10.1038/s41586-022-04965-x>

~600M SNPs (representing 7% of all possible human SNPs)

~60M indels

~1M structural variants

~3M microsatellites

Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Genomic Prediction from Whole Genome Sequence in Livestock: the 1000 Bull Genomes Project

B.J. Hayes^{1,2,3}, I.M. MacLeod^{3,4}, H.D. Daetwyler^{1,2,3}, P.J. Bowman^{2,3}, A.J. Chamberlain^{2,3}, C.J. Vander Jagt^{2,3}, A. Capitan^{5,6}, H. Pausch⁶, P. Stothard⁷, X. Liao⁷, C. Schrooten⁸, E. Mullaart⁸, R. Fries⁶, B. Guldbrandtsen⁹, M.S. Lund⁹, D.A. Boichard⁵, R.F. Veerkamp¹⁰, C.P. VanTassell¹¹, B. Gredler¹², T. Druet¹³, A. Bagnato¹⁴, J. Vilkki¹⁵, D.J. deKoning¹⁶, E. Santus¹⁷, and M.E. Goddard^{2,3,4}.



Simple Genetics: Mutations

- **Base change every 500-1000 bases**
 - AAGTACATGGC
 - AAATACATGGC
 - AAGTATATGGC
 - AAGTACATCGC
 - AAGTACATGGA
- **Mutations within genes:**
 - No effect
 - Altered amino acid (hence protein)
- **Mutations outside genes**
 - No effect
 - Altered expression of nearby gene



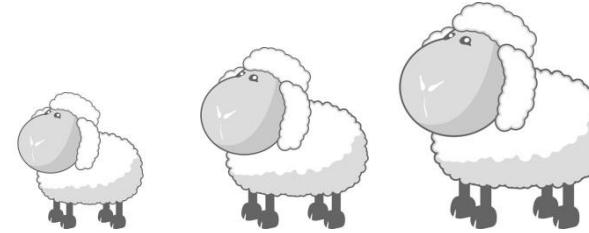
SNPs and SNP Chips

- **SNP = Single Nucleotide Polymorphism**
 - Specific base where animals differ (G,C,A,T)
- **Typical SNP chips ~50,000 SNPs**
 - 50k simultaneous genotypes
- **Typical genome ~ 3,000,000,000 bases**
 - = 1,000,000 pages of info
 - = 2,000 airport blockbusters
 - Info every 60,000 bases
- **50k SNP chips captures ~ 1% of all SNPs**



“Mutation-Dependent” Genetics

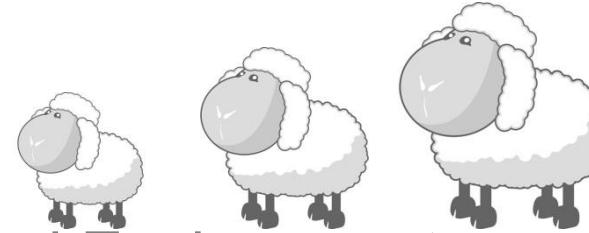
- Consider a population:
 - Each individual is different
 - Differences due to **Genetics and Environment**
- Consider genetics:
 - All individuals have the **same genes**
 - But they have different **variants** of each gene





“Mutation-Dependent” Genetics

- Consider a population:
 - Each individual is different
 - Differences due to Genetics and Environment
- Consider genetics:
 - All individuals have the same genes
 - But they have different variants of each gene
- **Consider different traits:**
 - **For some: between-animal variation due to variants at a few genes**
 - **For others: variants at many genes are important**





Motivation for genome-wide association studies

Concept is “simple”:

- Find genes related to the traits of interest
- Get the insight into genetic architecture of the trait

Several strategies exists; linkage analysis, association mapping, ...

With availability of genome-wide dense SNP chips

- Test associations between SNP and phenotype → GWAS
- Where significant associations → SNP in LD with QTL

Genetic Models

- Consider a single locus (or SNP)

Genotypes: tt tT TT

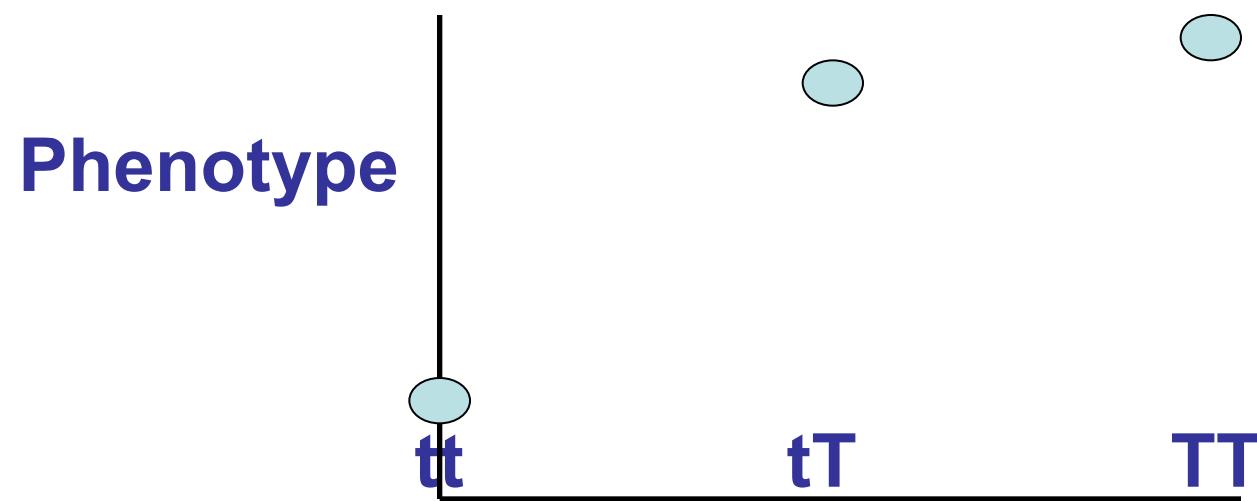
freq.(t) = 1-p = q; freq.(T) = p



Genetic Models

- Consider a single locus (or SNP)

Genotypes: tt tT TT
 $\text{freq.}(t) = 1-p = q; \text{ freq.}(T) = p$

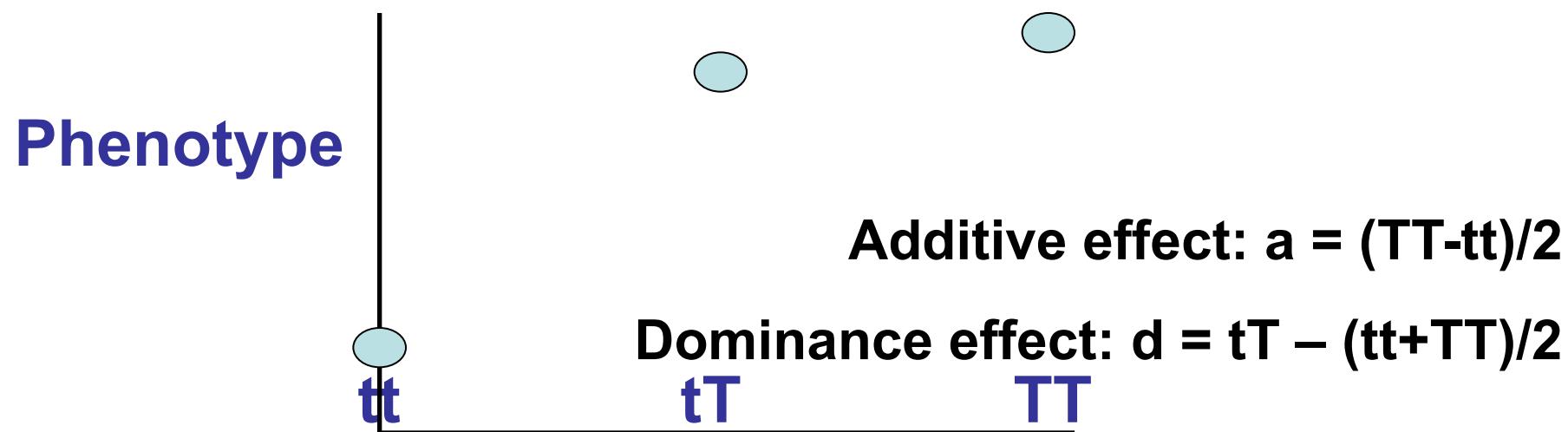




Genetic Models

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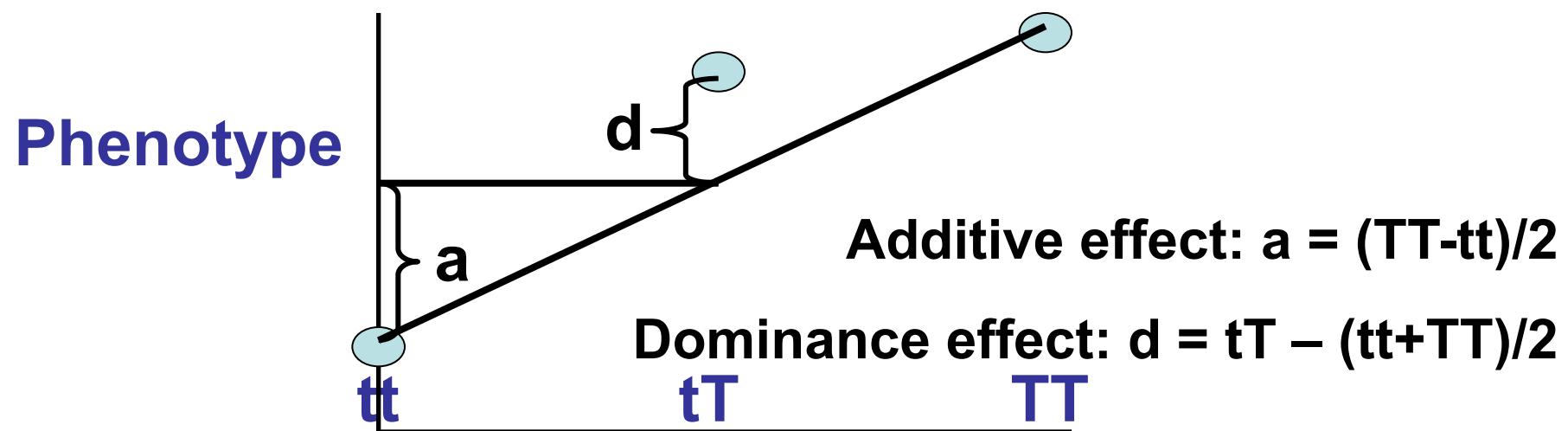




Genetic Models

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Genotypes: tt tT TT
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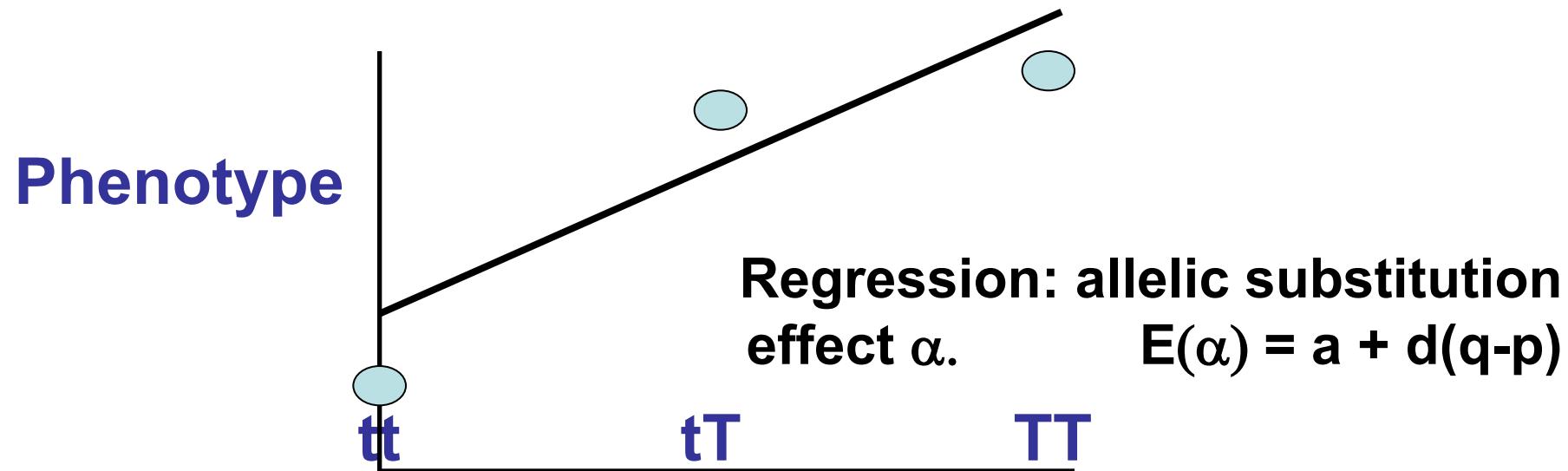




Genetic Models

- Consider a single locus (or SNP)

Genotypes: tt tT TT
 $\text{freq.}(t) = 1-p = q; \text{ freq.}(T) = p$



Genetic Models

- Consider many loci

$$P = \mu + G + E$$



Genetic Models

- Consider many loci

Phenotype Environment

$$\downarrow \qquad \qquad \qquad \swarrow$$
$$P = \mu + G + E$$

Genotype = $\sum g_j$ (j=1,m loci) (i=1,n animals)



Genetic Models

- Consider many loci

Phenotype Environment

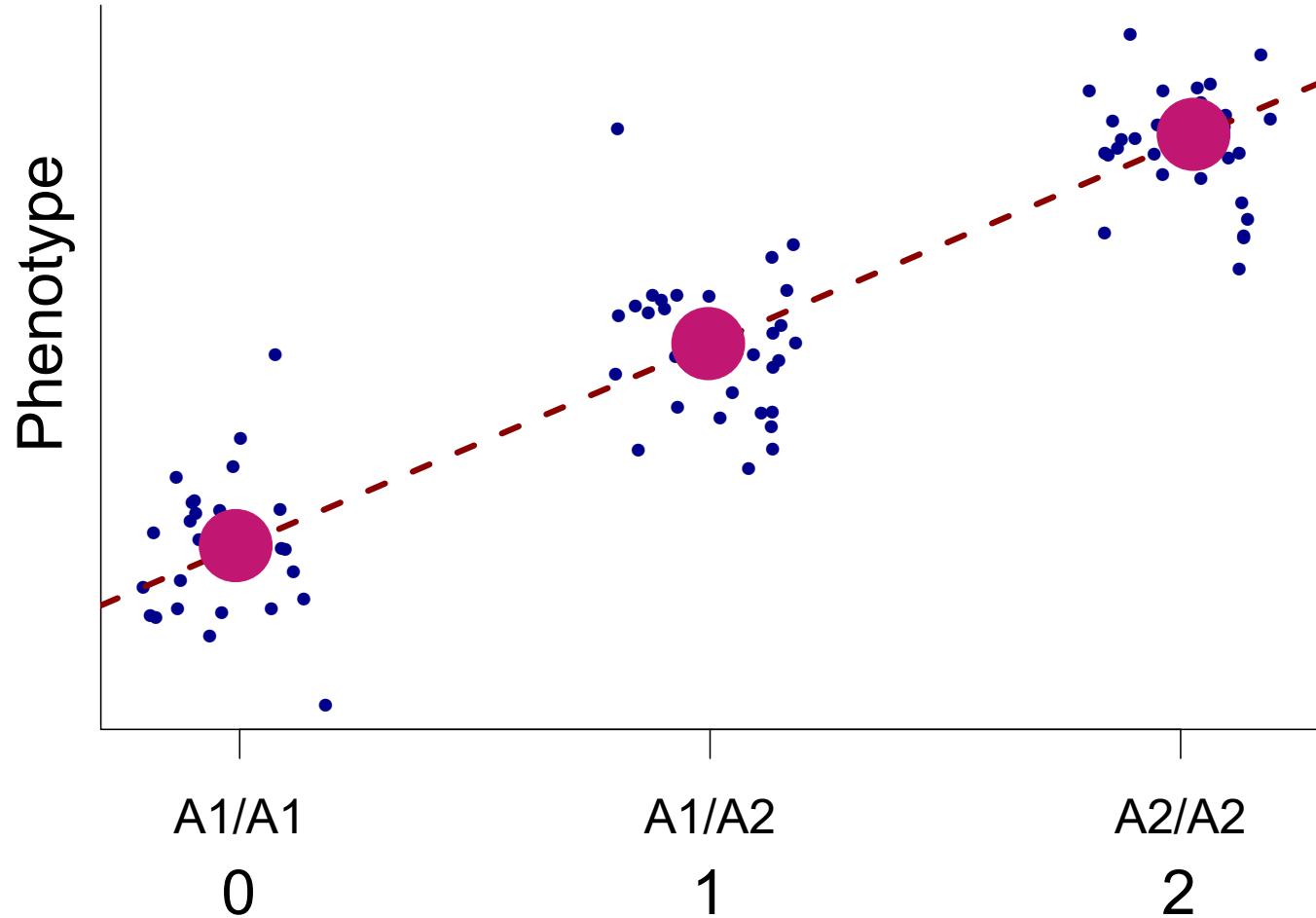
$$P = \mu + G + SNPj + E$$

Genotype = Σg_j (j=1,m loci) (i=1,n animals)



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Basic Principal



Linear Mixed Models

- $\mathbf{Y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{W}\mathbf{c} + \dots$
- \mathbf{b} = vector of fixed effects, \mathbf{X} = design matrix
 - INCLUDES SNP
- \mathbf{u} = vector of random genetic effects, \mathbf{Z} = design ...
- \mathbf{c} = vector of another random effect
 - E.g. litter effect, maternal, QTL, ...
- Estimate \mathbf{b} , \mathbf{u} , \mathbf{c} , etc and variance components
 - Estimated \mathbf{u} = Estimated Breeding Values (EBV)



Linear Mixed Models

- $\mathbf{Y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{W}\mathbf{c} + \dots$
- **b = vector of fixed effects, X = design matrix**
 - INCLUDES SNP
- **u = vector of random genetic effects, Z = design ...**
- **c = vector of another random effect**
 - E.g. litter effect, maternal, QTL, ...
- **Estimate b, u, c, etc and variance components**
 - Estimated u = Estimated Breeding Values (EBV)
- **50,000 SNPs => specialised software needed**



SNP Association Issues

- Linkage Disequilibrium (LD)
- Population Structure
- Multiple Testing



Definitions of LD

- LD is required for both linkage and linkage disequilibrium mapping
- Difference:
 - linkage analysis mapping considers the within family LD
 - extends for 10s of cM and broken down after only a few generations
 - LD mapping requires a marker allele to have within population LD with a QTL allele
 - association must have persisted across multiple generations as a property of the population
 - Which means marker and QTL must be very close

Definitions of LD

| | | Marker A | | |
|----------|------|----------|-----|------|
| | | A1 | A2 | Freq |
| Marker B | B1 | 0.4 | 0.1 | 0.5 |
| | B2 | 0.1 | 0.4 | 0.5 |
| | Freq | 0.5 | 0.5 | |

$$\begin{aligned}
 D &= \text{freq}(A1_B1) * \text{freq}(A2_B2) - \text{freq}(A1_B2) * \text{freq}(A2_B1) \\
 &= 0.4 * 0.4 - 0.1 * 0.1 \\
 &= 0.15
 \end{aligned}$$

Definitions of LD

- Measuring LD (determines how dense markers need to be for LD mapping)

$$D = \text{freq}(A1_B1) * \text{freq}(A2_B2) - \text{freq}(A1_B2) * \text{freq}(A2_B1)$$

– highly dependent on allele frequencies

- not suitable for comparing LD at different sites

$$r^2 = D^2 / [\text{freq}(A1) * \text{freq}(A2) * \text{freq}(B1) * \text{freq}(B2)]$$



Definitions of LD

| | | Marker A | | |
|----------|------|----------|-----|------|
| | | A1 | A2 | Freq |
| Marker B | B1 | 0.4 | 0.1 | 0.5 |
| | B2 | 0.1 | 0.4 | 0.5 |
| | Freq | 0.5 | 0.5 | |

$$D = 0.15$$

$$r^2 = D^2 / [\text{freq}(A1) * \text{freq}(A2) * \text{freq}(B1) * \text{freq}(B2)]$$

$$r^2 = 0.15^2 / [0.5 * 0.5 * 0.5 * 0.5]$$

$$= 0.36$$

Definitions of LD

- If we assume two loci: one is marker and the other QTL
- r^2 between the two can give the proportion of QTL variance which can be observed at the marker
 - e.g. if variance due to a QTL is 300kg^2 , and r^2 between marker and QTL is 0.2, variation observed at the marker is 60kg^2 .
- The power of LD mapping to detect QTL
 - sample size needs be increased by $1/r^2$ to have the same power as an experiment observing directly the QTL

Definitions of LD

- Multi-locus measures of LD
 - r^2 is easy to calculate, useful and widely used
 - Takes two loci at a time
 - Does not tell us of the causes of LD



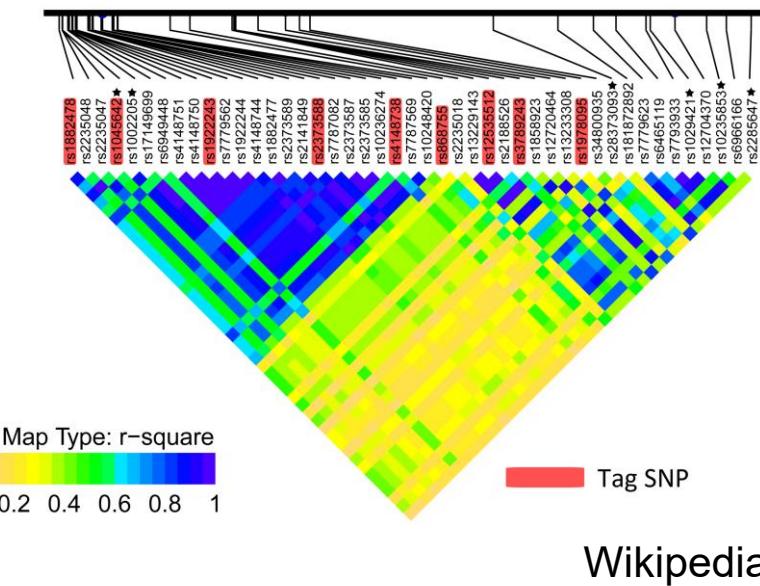
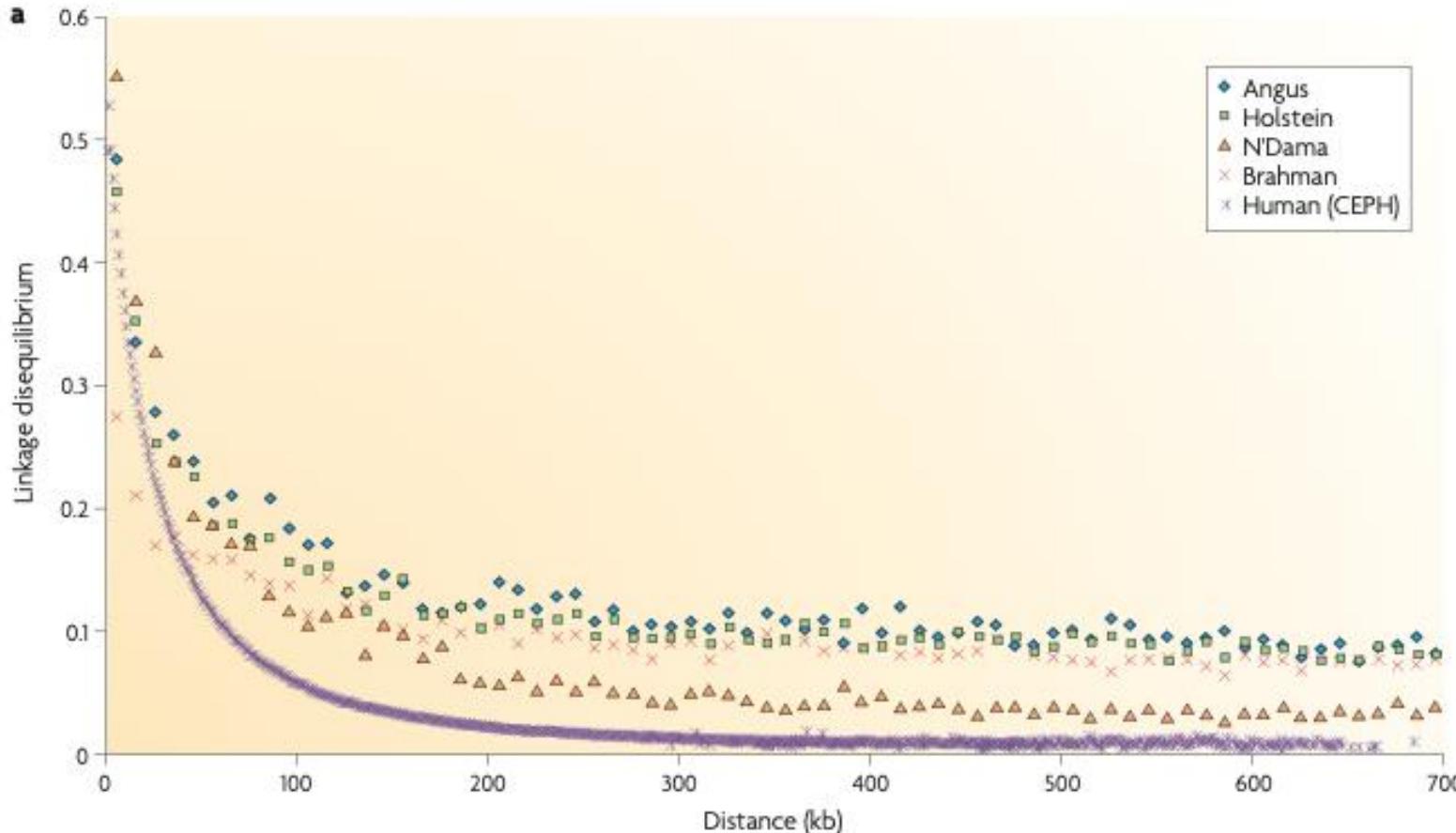
Causes of LD

- **Migration**
 - LD artificially created in crosses
 - large when crossing inbred lines
 - small in cross breeding of breeds that are similar in gene frequencies
 - Transitional- disappears after a number of generations
- **Selection**
 - Selective sweeps
- **Population size**
 - Livestock have small effective pop. Size >> High LD
 - Humans have large effective pop. Size >> Low LD



Foundation is linkage disequilibrium (LD)

Non-random association of alleles between loci

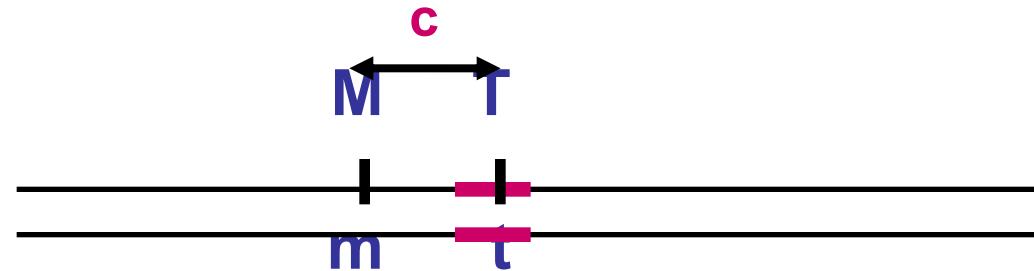


Wikipedia



SNP Association Issues

- LD



LD = correlation (r) M/m with T/t

$r \downarrow$ as $c \uparrow$

$E(r | c)$ ~ age of mutations
~ effective population size
~ population history, including selection

At population level: SNP & Causative mutation must be close

SNP Association Issues

- **Population Structure (stratification)**
 - Cause of MANY false positive associations

SNP Association Issues

- causes of Population Structure:
 - Anything!!
 - Breed
 - Families
 - Selection
 - Biased sampling
 - Often hard to spot

SNP Association Issues

- Population Structure solution
 - Use known pedigree or SNP Genotypes to detect & remove structure
- $Y = Xb + Zu + \beta \cdot SNP$

- β = regression (as before)
- u = vector of genetic effects
 - $Var(u) \sim A\sigma^2_a$ or $K\sigma^2_a$
 - Where K is matrix of covariances of genotypes
 - Where A is matrix of covariances by pedigree

SNP Association Issues

- **Multiple Testing**
 - E.g. 50k or 750k statistical tests
 - Many “significant” results by chance alone
 - The most significant results have upwards bias
 - Validate results in independent sample to get unbiased



SNP Association Issues

- **Multiple Testing**
 - E.g. 50k or 750k statistical tests
 - Many “significant” results by chance alone
- **Solution: stringent thresholds**
 - Bonferroni: e.g. $0.05/n \rightarrow 1 \times 10^{-6}$ for 50k SNPs
 - Empirical thresholds from permutation

Need large studies to have power to declare associations significant (unless SNP effect large)

SNP Association Examples

- Same as for heritabilities
 - FIT SNP as fixed effect as well
- Additive variance due to SNP = $2pq[a + d(q-p)]^2$
- SNP heritability = $2pq[a + d(q-p)]^2/V_p$

- **Association analysis**
 - Simple Mixed Model (animal model) – as in h^2 analyses:

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

- Add one or all SNPs:

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{X}\mathbf{b} + \beta_i m_i + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

$$\mathbf{y} = \boldsymbol{\mu} + \mathbf{X}\mathbf{b} + \sum \beta_i m_i + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

GWAS

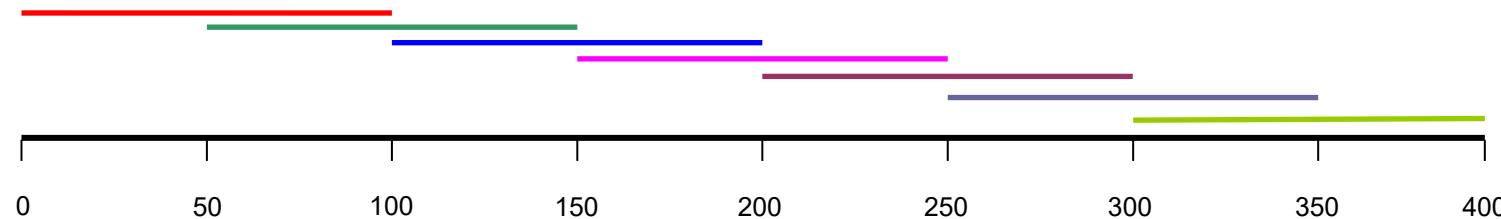
Relationship
matrix **G**

Genomic selection



Regional Heritability Mapping (RHM)

- Variance component approach
- Fit joint effects of all loci within a genomic region
- Each chromosome is divided into windows of a pre-defined number of SNPs:
 - In our case, 100 SNPs and window shifted every 50 SNPs





Regional Heritability Mapping (RHM)

$$y = Xb + Za + Zw + e$$

overall genetic effect

regional combined genetic effect

$$h^2 = \frac{(\sigma_a^2 + \sigma_w^2)}{(\sigma_a^2 + \sigma_w^2 + \sigma_e^2)}$$

Total h^2

$$h_w^2 = \frac{\sigma_w^2}{(\sigma_a^2 + \sigma_w^2 + \sigma_e^2)}$$

Regional h^2



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GWAS methods

Single-marker regression

Multiple-marker model

- Multiple regressions
- Fit as a random effects via Bayesian regression methods
- Borrowed from GS
- Note equivalence; GWAS by GBLUP



Multiple testing correction

Bonferroni correction

- Significance level / Number of tests
- Conservative
- For 50 K SNP: $0.05/50000 = 10^{-6}$
- For 1 M SNP: $0.05/10^6 = 5 \times 10^{-8}$

Other options

- Limiting the false discovery rate (FDR)
- Permutation testing
- Effective number of independent tests
- Bayesian approach
- ...



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Importance of quality control

Genotype call rate (SNP, individual)

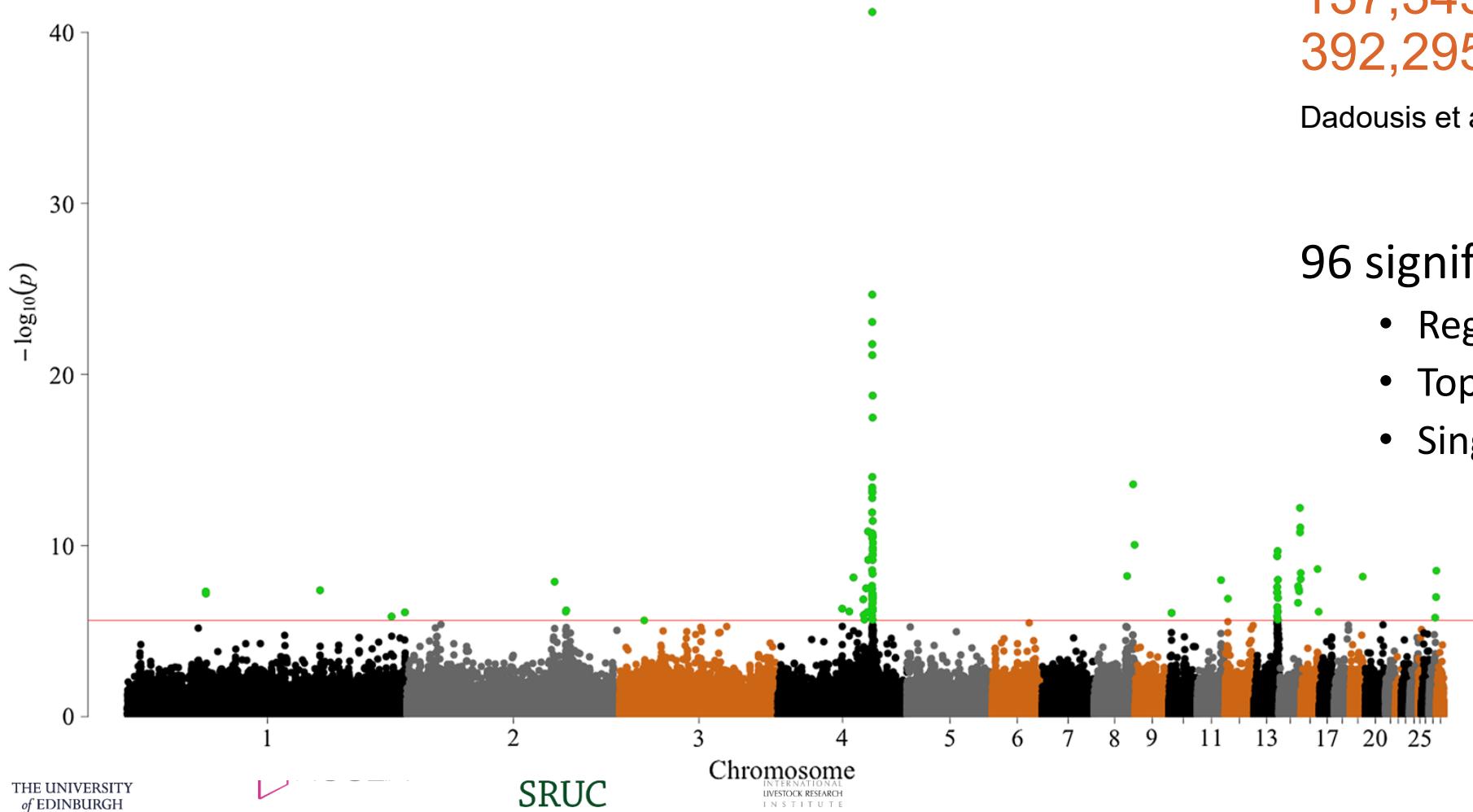
Minor allele frequency

Departure from Hardy–Weinberg equilibrium

Don't forget QC of phenotypes!



Results: Manhattan plot



137,343 broiler chickens
392,295 imputed SNP

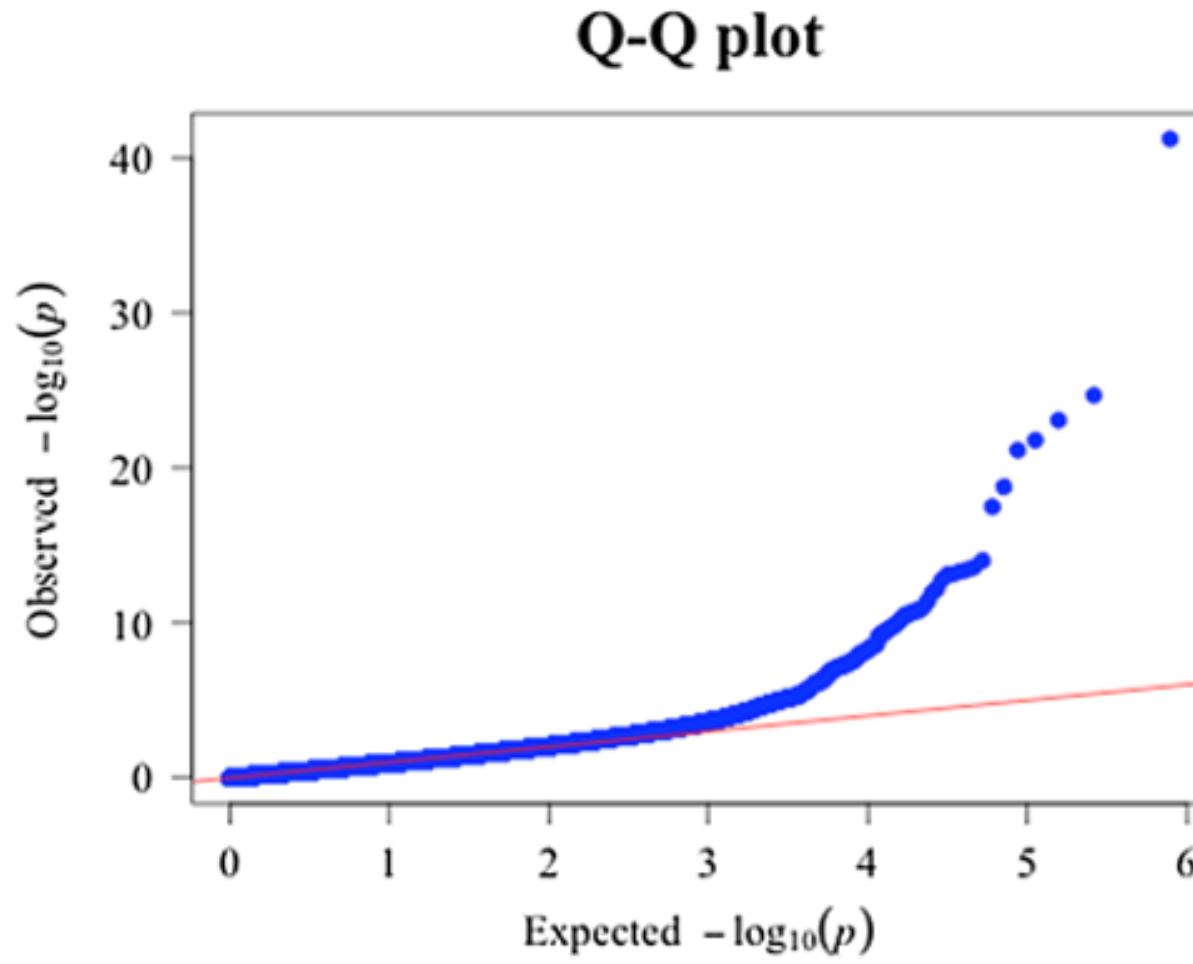
Dadousis et al., 2021

96 significant SNP in 25 regions

- Regions explained 30% V_A
- Top region 4.37% V_A
- Single top SNP 1.9% V_A

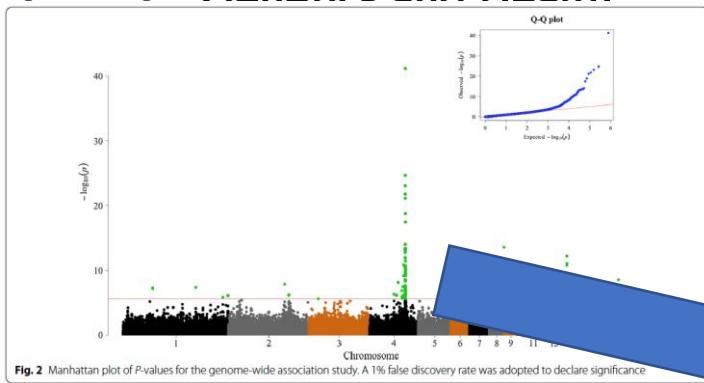


Results: Q-Q (quantile-quantile) plots





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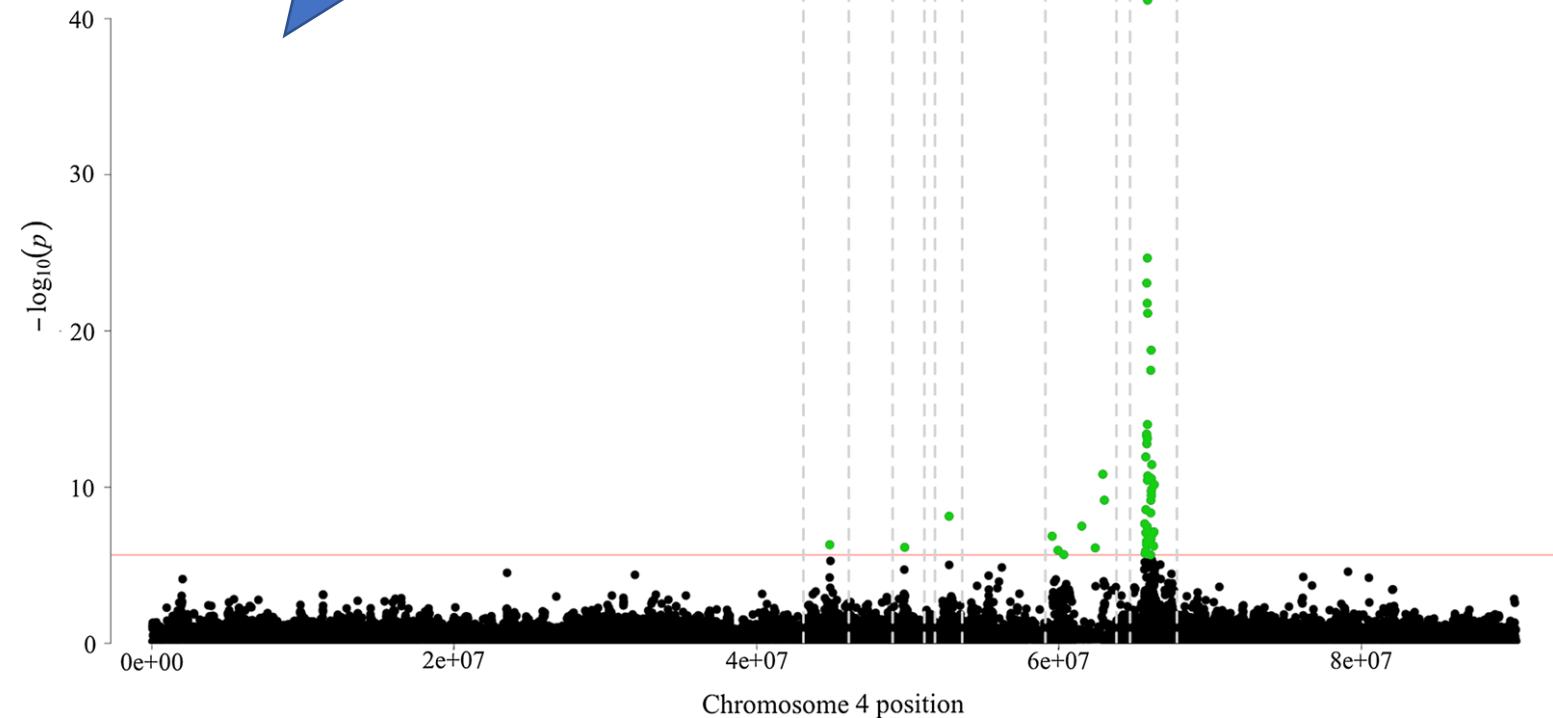


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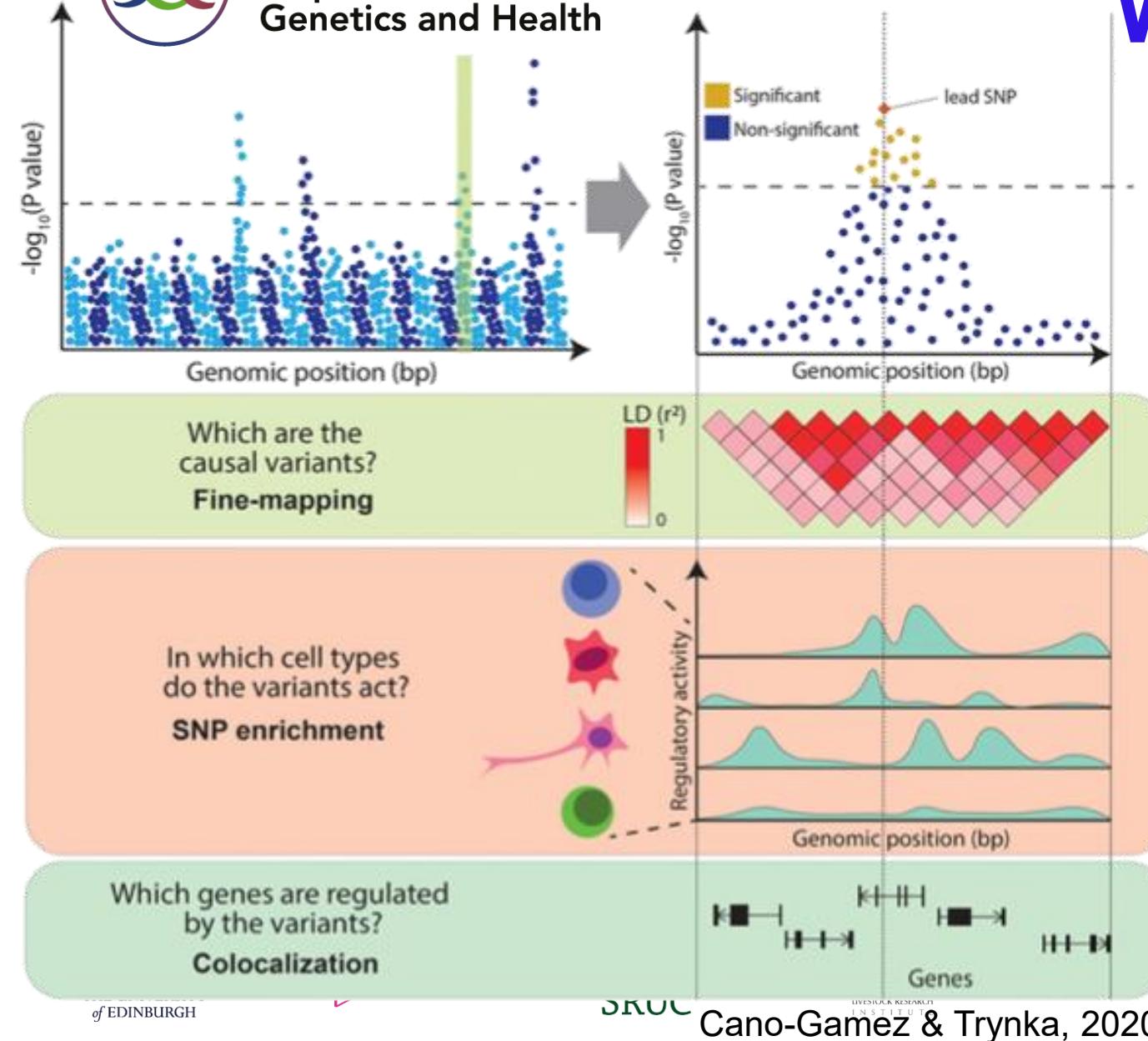
Results: Manhattan plot

GGA4





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What's after GWAS?

GWAS association \neq causal variant
Biological validation and causal variants?

Replications? Fine mapping?

Functional analysis? eQTL? Genome editing?

Is GWAS able to enrich the genomic evaluations?



I will leave it up to you

What is the future of GWAS?

Five Years of GWAS Discovery

Peter M. Visscher,^{1,2,*} Matthew A. Brown,¹ Mark I. McCarthy,^{3,4} and Jian Yang⁵

10 Years of GWAS Discovery: Biology, Function, and Translation

Peter M. Visscher,^{1,2,*} Naomi R. Wray,^{1,2} Qian Zhang,¹ Pamela Sklar,³ Mark I. McCarthy,^{4,5,6}
Matthew A. Brown,⁷ and Jian Yang^{1,2}

Evidence for and localization of proposed causative variants in cattle and pig genomes

Martin Johnsson^{1*} and Melissa K. Jungnickel²

Status and prospects of genome-wide association studies in plants

Laura Tibbs Cortes¹ | Zhiwu Zhang² | Jianming Yu¹

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Animal QTL data base



Cattle QTL

There are **163,725** QTL from **1,069** publications curated into the database. Those QTL represent **685** different traits (see [data summary](#) for details).



Catfish QTL

There are **0** QTL from **0** publications curated into the database. Those QTL represent **0** different traits (see [data summary](#) for details).



Chicken QTL

There are **15,475** QTL from **362** publications curated into the database. Those QTL represent **442** different traits (see [data summary](#) for details).



Goat QTL NEW

There are **64** QTL from **3** publications curated into the database. Those QTL represent **21** different traits (see [data summary](#) for details).



Horse QTL

There are **2,473** QTL from **99** publications curated into the database. Those QTL represent **62** different traits (see [data summary](#) for details).



Pig QTL

There are **33,540** QTL from **745** publications curated into the database. Those QTL represent **704** different traits (see [data summary](#) for details).



Rainbow Trout QTL

There are **1,372** QTL from **17** publications curated into the database. Those QTL represent **22** different traits (see [data summary](#) for most recent updates).



Sheep QTL

There are **3,752** QTL from **201** publications curated into the database. Those QTL represent **274** different traits (see [data summary](#) for most recent updates).



Top 15 QTL/associations

| Traits | Number of QTL |
|---|---------------|
| Age at puberty | 10,623 |
| Scrotal circumference | 10,457 |
| Milk fat percentage | 8,117 |
| Milk fat yield | 6,957 |
| Milk protein percentage | 4,999 |
| Milk C14 index | 4,847 |
| Milk kappa-casein percentage | 4,836 |
| Metabolic body weight | 4,275 |
| Milk yield | 3,835 |
| Percentage normal sperm | 3,596 |
| Calving ease | 3,540 |
| Average daily gain | 3,504 |
| Milk myristoleic acid content | 3,313 |
| Milk protein yield | 3,095 |
| Milk glycosylated kappa-casein percentage | 2,753 |

<https://www.animalgenome.org/cgi-bin/QTLdb/index>



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Examples



Change of perspective

Infinitesimal model:

- Infinite number of additive loci; each with a small effect

Finite loci model:

- Finite amount of DNA and genes
- How many?
- Small/big effect?



Basic statistical model

Phenotype = $f(\text{genotype}, e)$

$$y_i = \mu + b x_i + e_i$$

Simple but many analysis

- 50 K SNP we run 50 K regressions, 100 K, 1M, ...

Put in matrix notation

$$\begin{aligned} \mathbf{y} &= \mathbf{1}\mu + \mathbf{x}\mathbf{b} + \mathbf{e} \\ \mathbf{y} &= \mathbf{X}\mathbf{b} + \mathbf{e} \end{aligned}$$



Relatedness and population structure

Cause of spurious associations

Account for it in a model:

- As a random effect;
 - Relationship matrix (A , G , ?)
- As a covariates;
 - Subpopulations (structured association; STRUCTURE)
 - PCA analysis (top PCs)

$$\begin{aligned}y &= Xb + Za + e \\Var(a) &= G\sigma_a^2 \\Var(e) &= I\sigma_e^2\end{aligned}$$