Genome-Wide Association Study

02-710 Computational Genomics

Seyoung Kim

Overview

- How can we identify the genetic loci responsible for determining phenotypes?
  - Linkage analysis
    - Data are collected for family members
    - Difficult to collect data on a large number of families
    - Effective for rare diseases
    - Low resolution on the genomes due to only few recombinations
      - » a large region of linkage

  - Genome-wide association studies
    - Data are collected for unrelated individuals
    - Easier to find a large number of affected individuals
    - Effective for common diseases, compared to family-based method
    - Relatively high resolution for pinpointing the locus linked to the phenotype

Overview

- Statistical methods for testing genotype/phenotype associations
  - Discrete-valued phenotype: case/control study
  - Continuous-valued phenotype: quantitative traits
  - Sparse regression method for considering all of the SNP markers
  - Multimarker association test

- Issues arising in GWAS
  - Genotype imputation
  - From common to rare variants
  - Epistasis for multiple interacting loci
  - Correcting for population structure

Population Genotype/Phenotype Data

Phenotype data

$$\boldsymbol{y} = \begin{pmatrix} y^1 \\ \vdots \\ y^N \end{pmatrix}$$

N individuals

Genotype data

J Loci

$$\boldsymbol{X} = \begin{pmatrix} x_1^1 & \cdots & x_J^1 \\ \vdots & & \vdots \\ x_1^N & \cdots & x_J^N \end{pmatrix}$$

N individuals

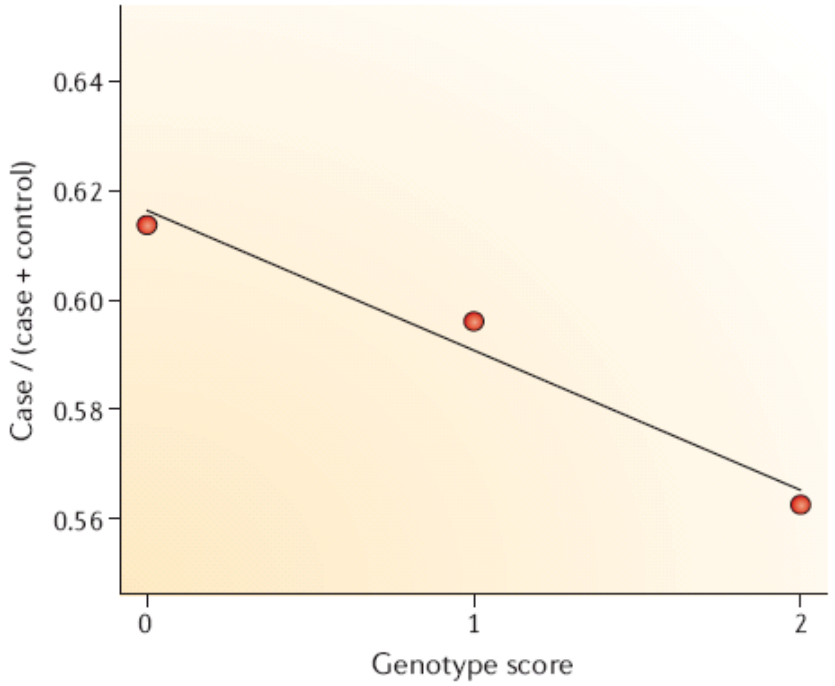
- 0 or 1 for case/control studies
  - e.g., healthy/diabetic
- Real-valued phenotypes
  - e.g., cholesterol level

Single SNP Association Test: Case/Control Study

- For each marker locus, find the 3x2 contingency table containing the counts of three genotypes

Genotype	Case	Control
AA	$N_{case,AA}$	$N_{control,AA}$
Aa	$N_{case,Aa}$	$N_{control,Aa}$
aa	$N_{case,aa}$	$N_{control,aa}$
Total	N_{case}	$N_{control}$

- χ^2 test with 2 df under the null hypothesis of no association



Genotype score = the number of minor alleles

Single SNP Association Analysis: Case/Control Study

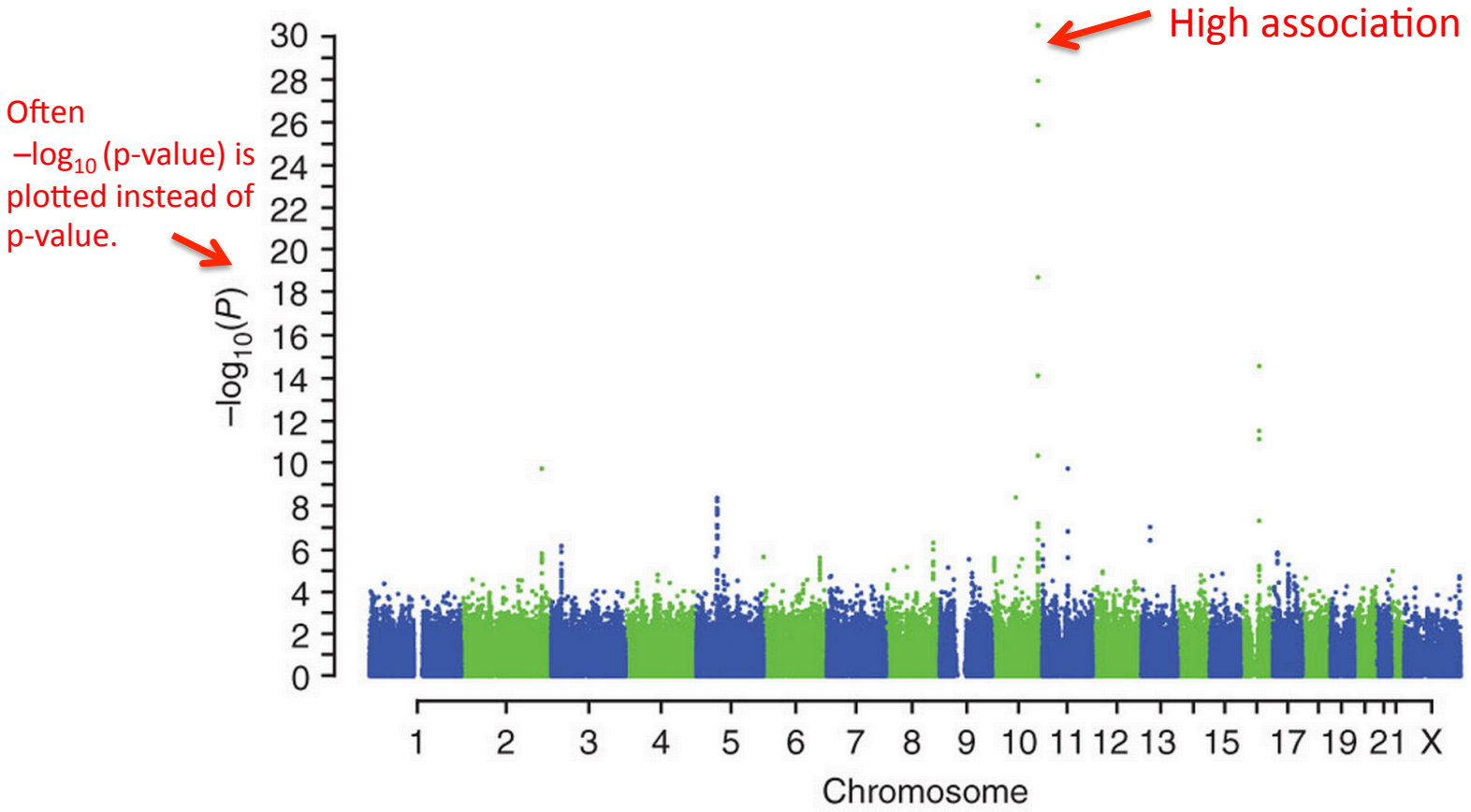
- Alternatively, assume the heterozygote risk is approximately between the two homozygotes
- Form a 2x2 contingency table. Each individual contributes twice from each of the two chromosomes.

Genotype	Case	Control
A	$G_{case,A}$	$G_{control,A}$
a	$G_{case,a}$	$G_{control,a}$
Total	$2xN_{case}$	$2xN_{control}$

- χ^2 test with 1df

Manhattan Plot of p-values from Breast Cancer GWAS

- Analysis of 582,886 SNPs for 3,659 cases with family history and 4,897 controls



Nature Genetics 42, 504-507 (2010)

Single SNP Association Test: Continuous-valued Traits

- Continuous-valued traits
  - Also called quantitative traits
  - Cholesterol level, blood pressure etc.

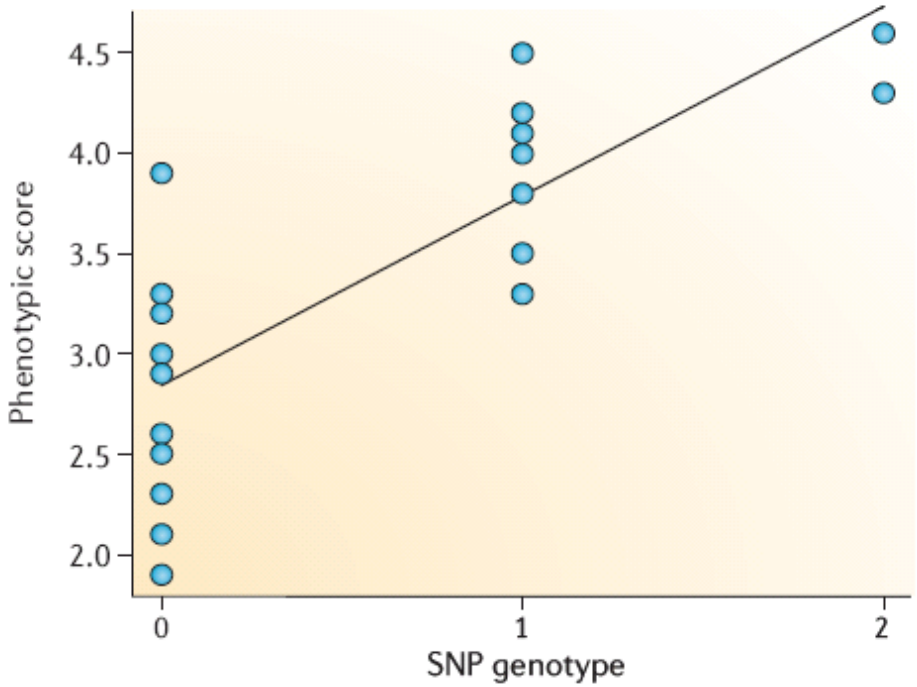
- For each locus, fit a linear regression at each locus

$$y_i = x_i\beta + \varepsilon$$

phenotype

genotype (number of minor alleles)

- *t*-test with null hypothesis "No associations, i.e., $\beta = 0$"



Genetic Model for Association

- Additive effect of minor allele, assuming effect size a for each minor allele
  - Major allele homozygote: 0
  - Heterozygote: a
  - Minor allele homozygote: $2a$

- Generalizing additive genetic models for heterozygotes: $a + a \times k$
  - k=1: dominant effect of the minor allele
  - k=0: no dominance
  - k=-1: dominant effect of the major allele

- Penetrance
  - Proportions of individuals carrying a particular allele that possess an associated trait
  - Alleles with high penetrance are easier to detect

Correcting for Multiple Testing

- What happens when we scan the genome of 1 million genetic markers for association with $\alpha = 0.05$?
  - 50,000 (=1 millionx0.05) SNPs are expected to be found significant just by chance
  - We need to be more conservative when we decide a given marker is significantly associated with the trait.

- Correction methods
  - Bonferroni correction
  - Permutation test

Bonferroni Correction

- If N markers are tested, we correct the significance level as $\alpha' = \alpha/N$
  - Assumes the N tests are independent, although this is not true because of the linkage disequilibrium.
  - Overly conservative for tightly linked markers

Permutation Procedure

- In order to generate the null distribution
  - Step 1: Set $N_{sig} = 0$
  - Step 2: Repeat 1:N_{perm}
    - Step 3a: Randomly permute the individuals in the phenotype data to generate datasets with no association (retain the original genotype)
    - Step 3b: Find the test statistics T_{perm} of SNPs using the permuted dataset
  - $T_1, \ldots, T_{Nperm}$ form a null distribution

- Compute the test statistic T using the original dataset and test with the above null distribution

This approach is computationally demanding because often a large N_{perm} is required.

Vector/Matrix Representation

- Sparse regression method to evaluate the effect of each SNP in the context of all other SNPs

$$\boldsymbol{y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon}$$

Phenotype data

Genotype data

$$\boldsymbol{y} = \begin{pmatrix} y^1 \\ \vdots \\ y^N \end{pmatrix} \quad N \text{ individuals}$$

J SNPs

$$\boldsymbol{X} = \begin{pmatrix} 1 & x_1^1 & \dots & x_J^1 \\ \vdots & \vdots & & \vdots \\ 1 & x_1^N & \dots & x_J^N \end{pmatrix} \quad N \text{ individuals}$$

$$\boldsymbol{\beta} = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \vdots \\ \beta_J \end{pmatrix}$$

Augmented input feature corresponding to β_0

  - Sparsity constraint: Only few SNPs are influencing the given phenotype and the rest of the SNPs have effect size 0, no multiple-hypothesis-testing problem

L1 Regularization (LASSO)

- A convex relaxation.

Constrained Form

$$\hat{\boldsymbol{\beta}} = \text{argmin}_{\beta} \|\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}\|^2$$

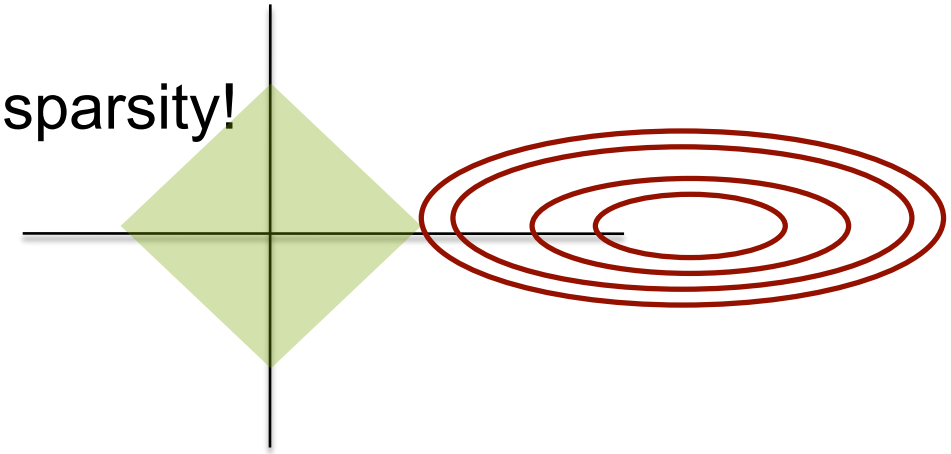
subject to:

$$\sum_{j=1}^{p} |\beta_j| \leq C$$

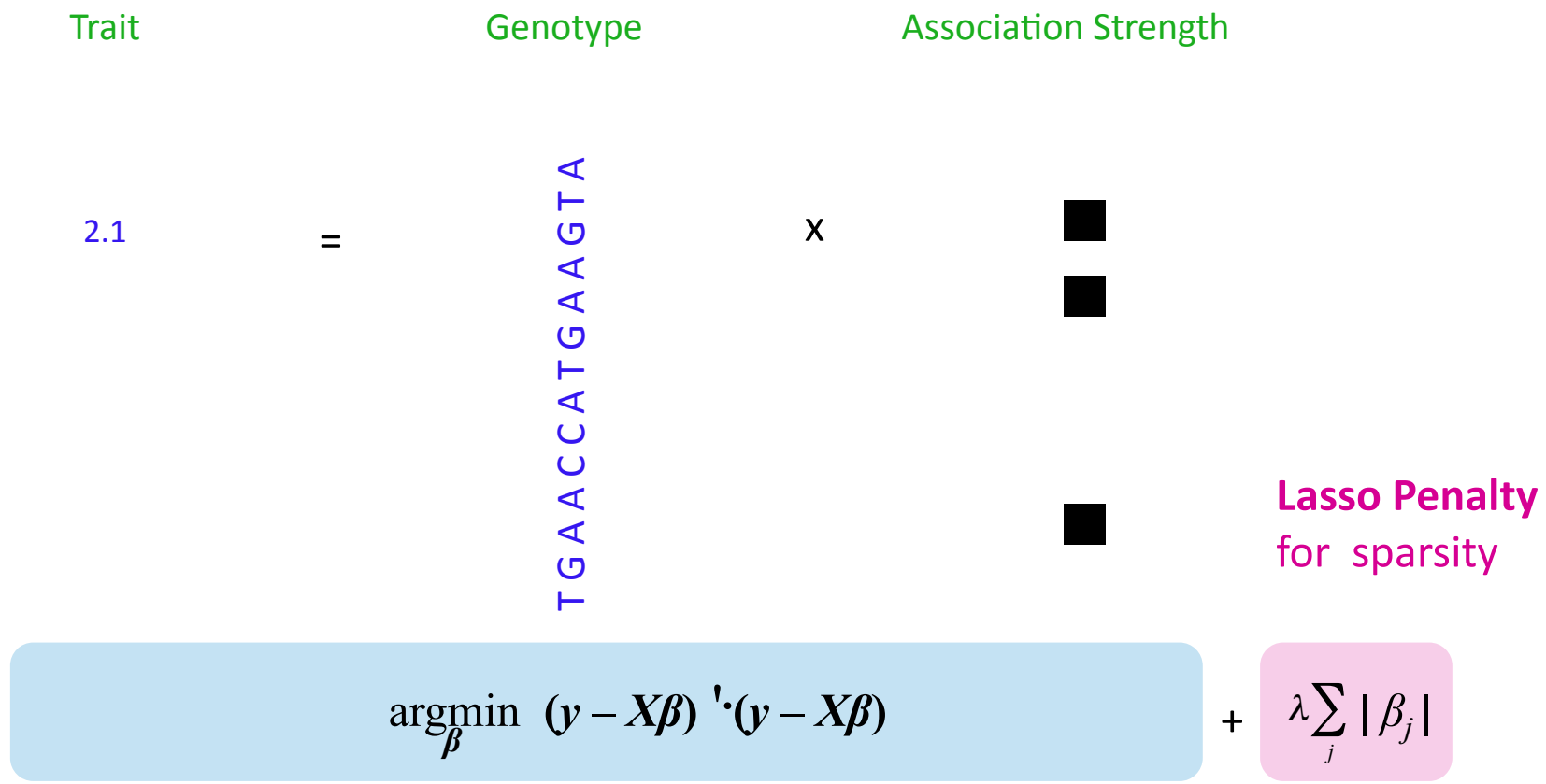
Lagrangian Form

$$\hat{\boldsymbol{\beta}} = \text{argmin}_{\beta} \|\mathbf{Y} - \mathbf{X}\boldsymbol{\beta}\|^2 + \lambda \|\boldsymbol{\beta}\|_1$$

- Still enforces sparsity!



Lasso for Reducing False Positives



Many zero associations (**sparse** results)

Tibshirani, Journal of Royal Statistical Society 1996
Wu et al., Bioinformatics 2009

Multi-marker (Haplotype) Association Test

- Idea: a haplotype of multiple SNPs is a better proxy for a true causal SNP than a single SNP

- Form a new allele by combining multiple SNPs for a haplotype

SNP A	SNP B
0	0
0	1
1	0
1	1

Auxiliary Markers for Haplotypes			
1	0	0	0
0	1	0	0
0	0	1	0
0	0	0	1

- Test the haplotype allele for association

Multi-marker Association Test

- Multi-marker approach can capture dependencies across multiple markers
  - SNPs in LD form a haplotype that can be tested as a single allele
  - Can achieve the higher power
    - Haplotypes are more powerful discriminators between cases and controls in disease association studies

- Challenge as the size of haplotype increases
  - Haplotype of *K* SNPs results in 2^K different haplotypes, but the number of samples corresponding to each haplotype decreases quickly as we increase *K*
  - Large *K* requires a large sample size

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  - From common to rare variants
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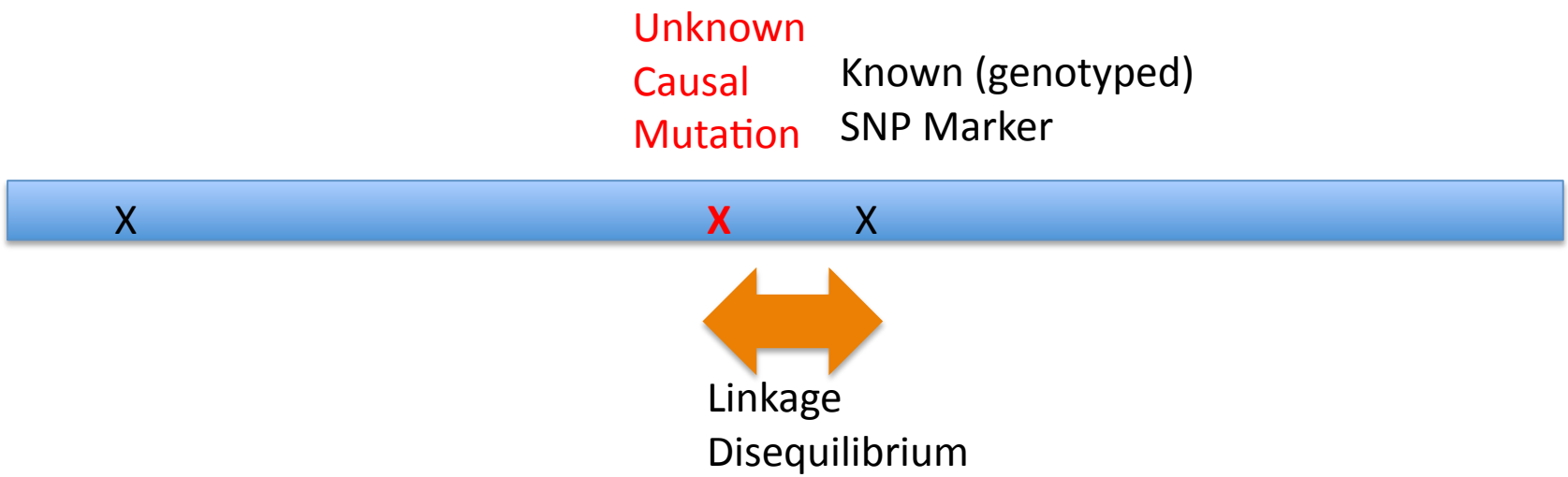
  - Correcting for population structure

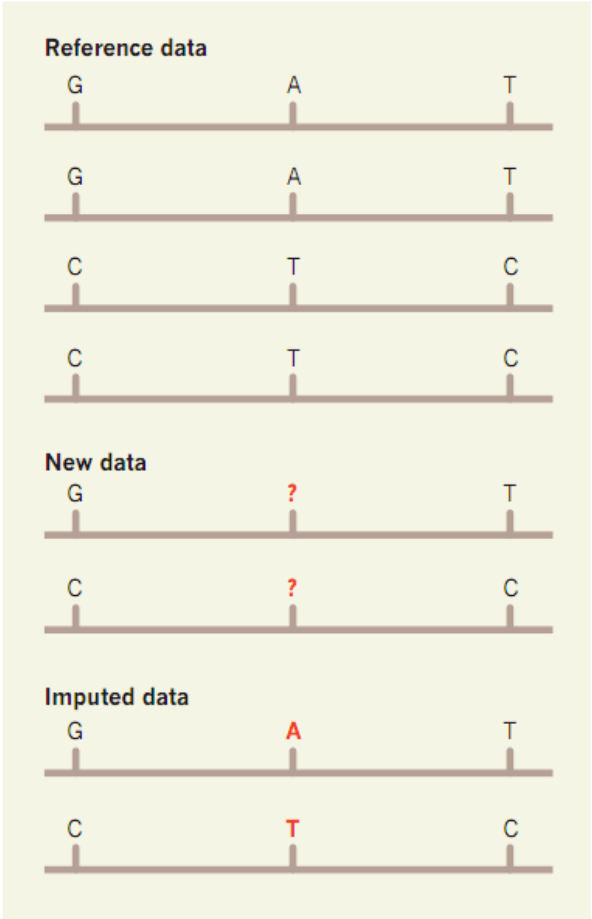
Causal Mutations and Genetic Markers

With SNP array data:



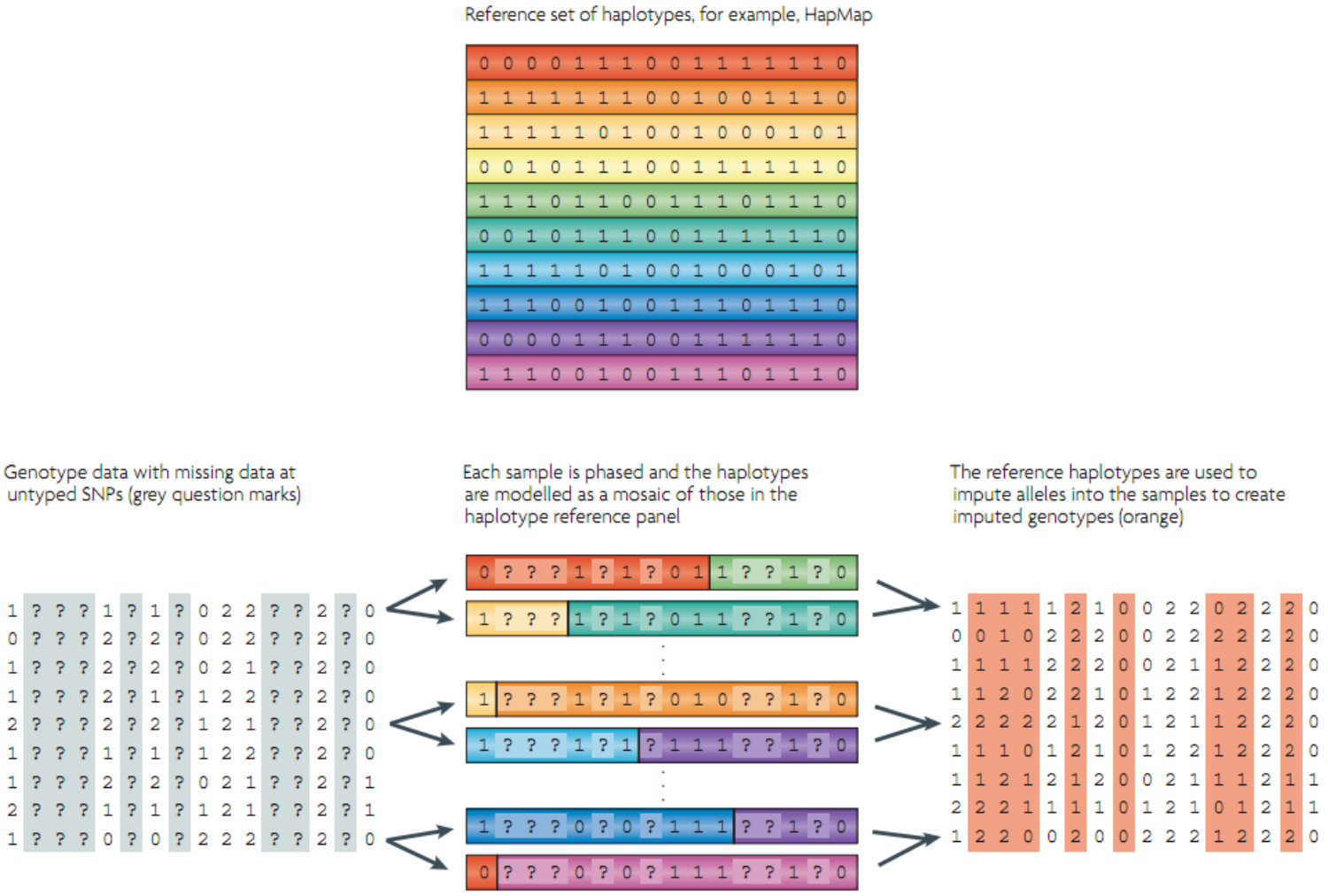
- What happens when SNP density increases?
- Fine mapping required to locate the causal mutation
- What happens with whole genome sequencing data?

Increasing SNP Density via Genotype Imputation



- Reference data: dense SNP data from HapMap III, or 1000 genome project
- New data: SNP data for individuals in a given study
- Data after imputation with the reference data (leverage LD!)

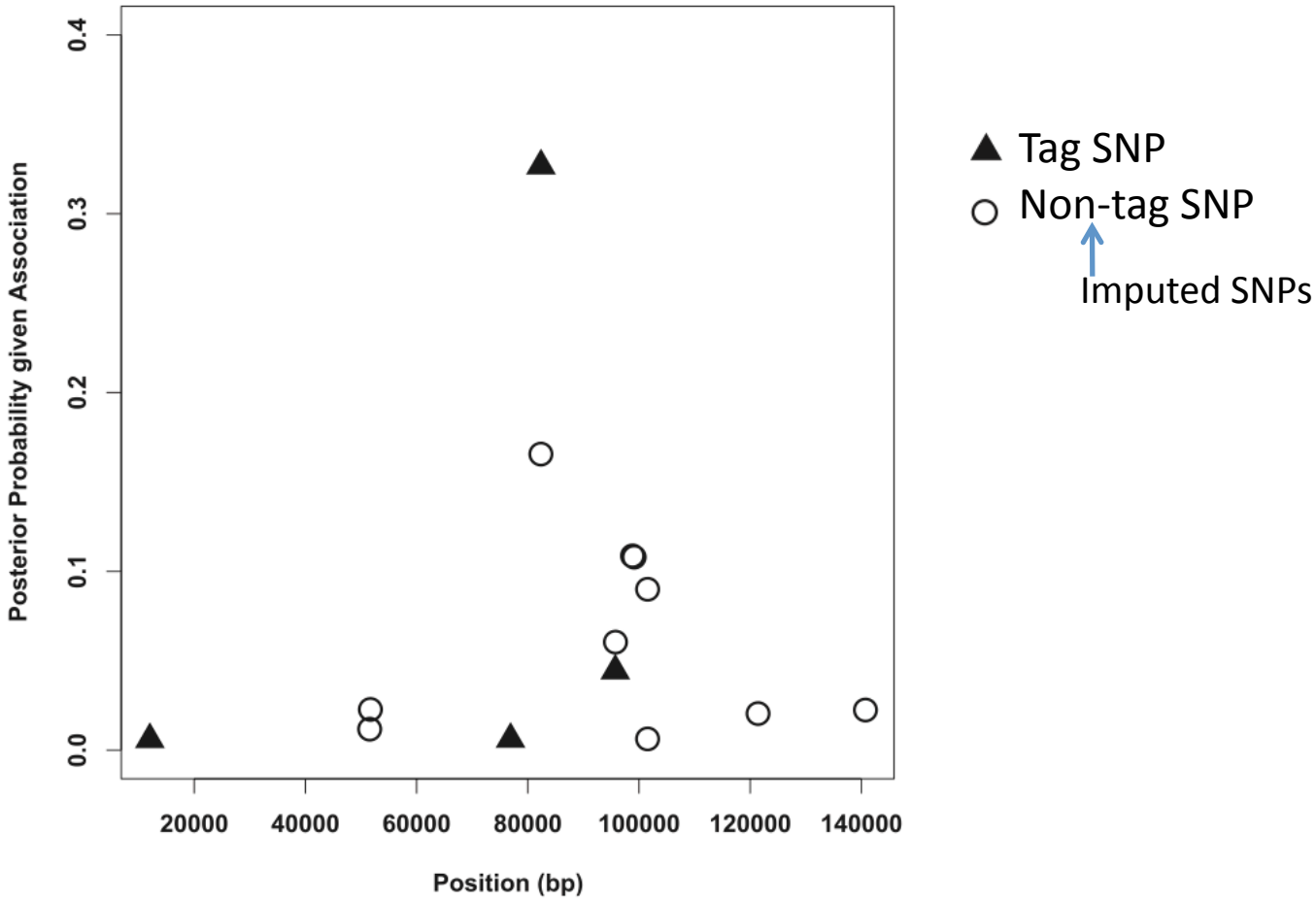
Genotype Imputation



PHASE can be used for imputation!

Imputation-Based Methods

(Servin & Stephens, 2007)



Common Variants vs. Rare Variants

- First-generation genome-wide association study (GWAS): common variant common disease hypothesis

- Common variants with minor allele frequency (MAF)>5%
  - dbGap: ~11 million SNPs
  - HapMap: 3.5 million SNPs
  - A successful GWAS requires a more complete catalogue of genetic variations

- Rare variants (MAF<0.5%), low-frequency variants (MAF:0.5%~5%)
  - Captured by sequencing with next-generation sequencing technology
  - Possibly significant contributors to the genetic architecture of disease
    - Causal variants are subject to negative selection

Associations to Rare Variants

- Often GWA studies are underpowered for functional rare variants

Common Variant Association

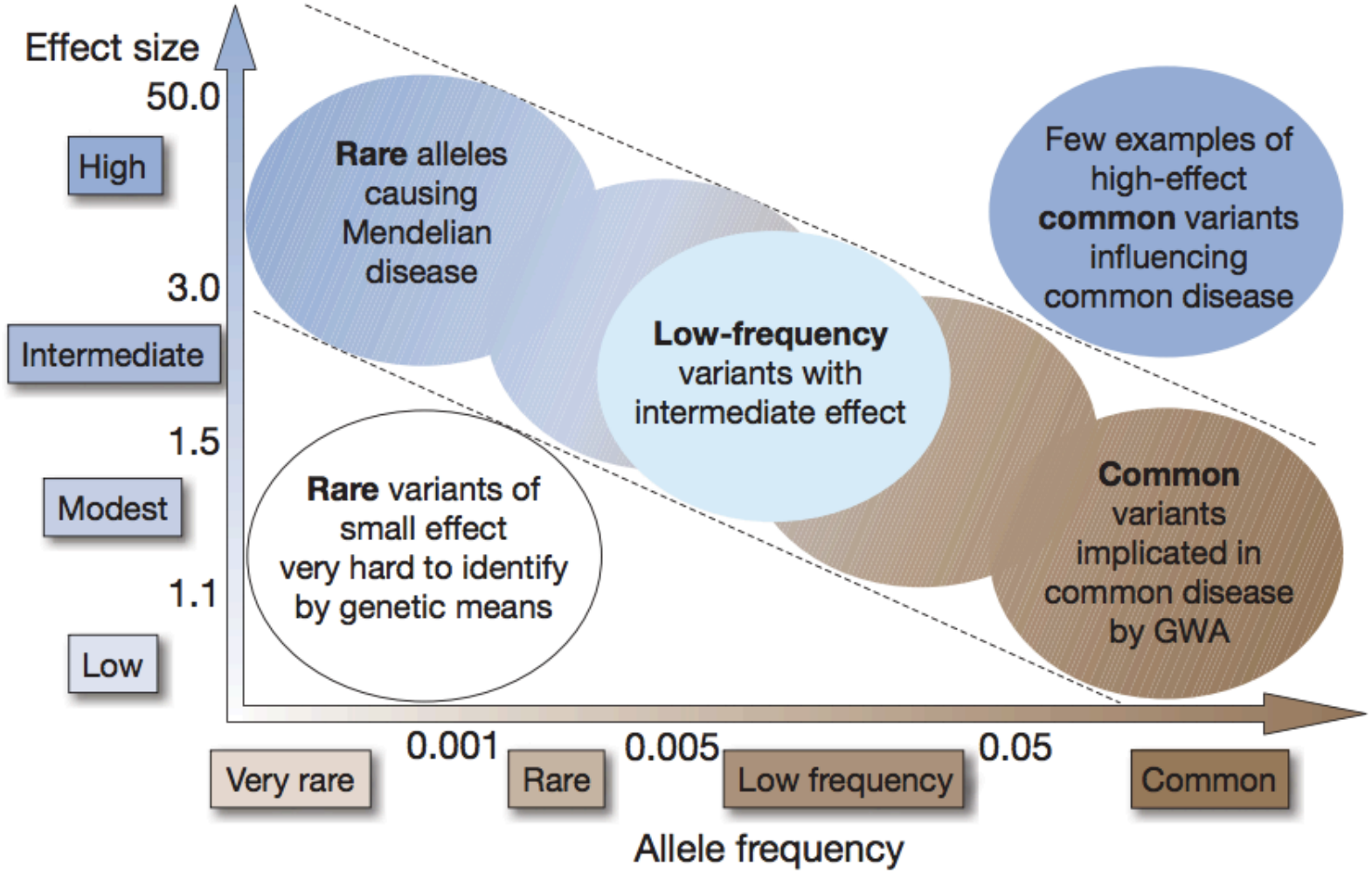
	Case	Control
Allele a	60	20
Allele A	40	80

Rare Variant Association

	Case	Control
Allele a	7	2
Allele A	93	98

- Common variant GWA approaches are appropriate only for common variants

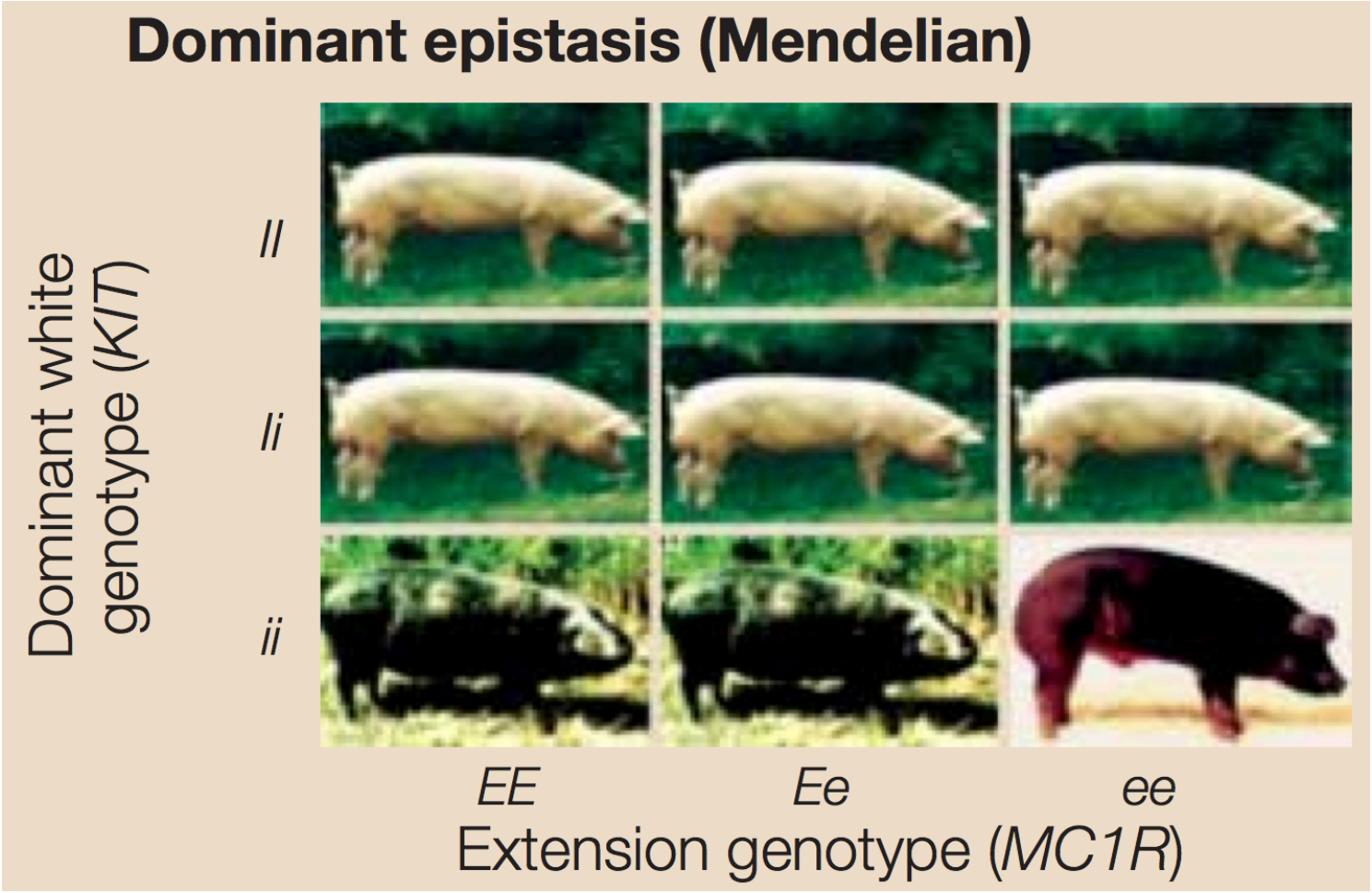
Feasibility of Identifying Disease Loci



Epistasis

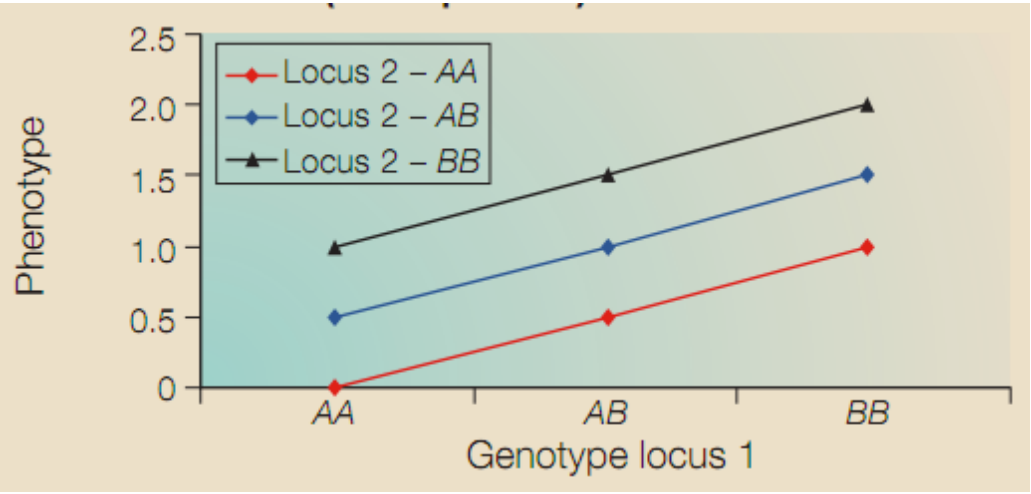
- Definition: The effect of one locus depends on the genotype of another locus
  - Epistatic effects vs. marginal effects

Epistasis for Mendelian Traits



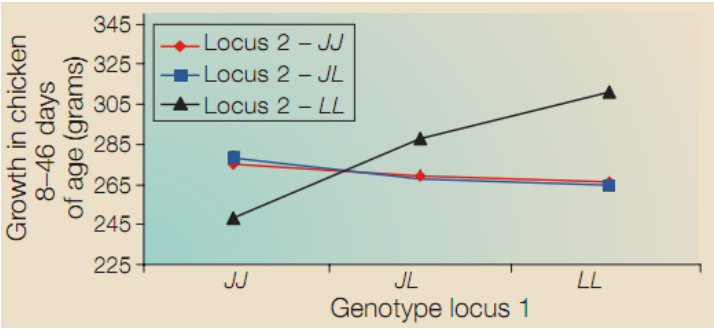
Carlborg & Haley, Nature Reviews Genetics 2004

When There is No Epistasis

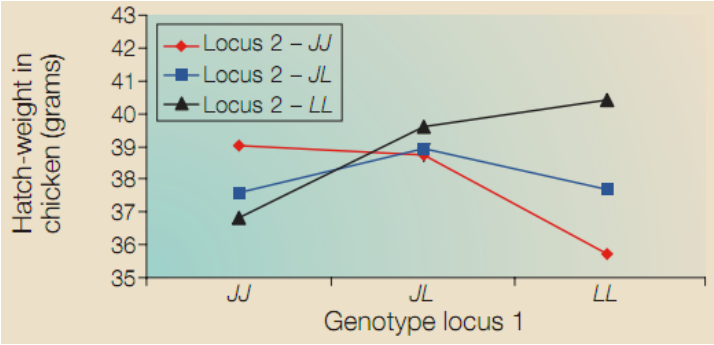


- Two additive (non-epistatic) loci
- The three lines run in parallel

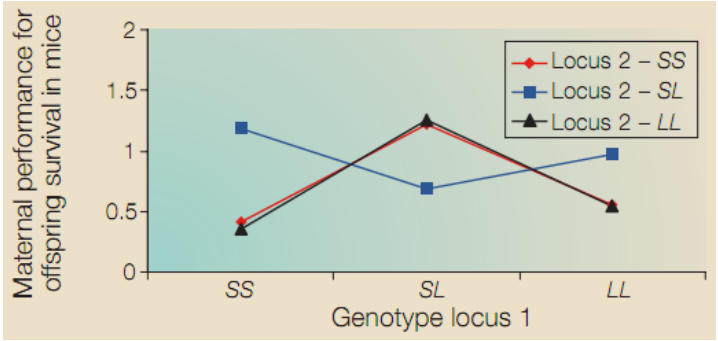
Epistasis Example



- Dominant epistasis
- One locus in a dominant way suppresses the allelic effects of a second locus

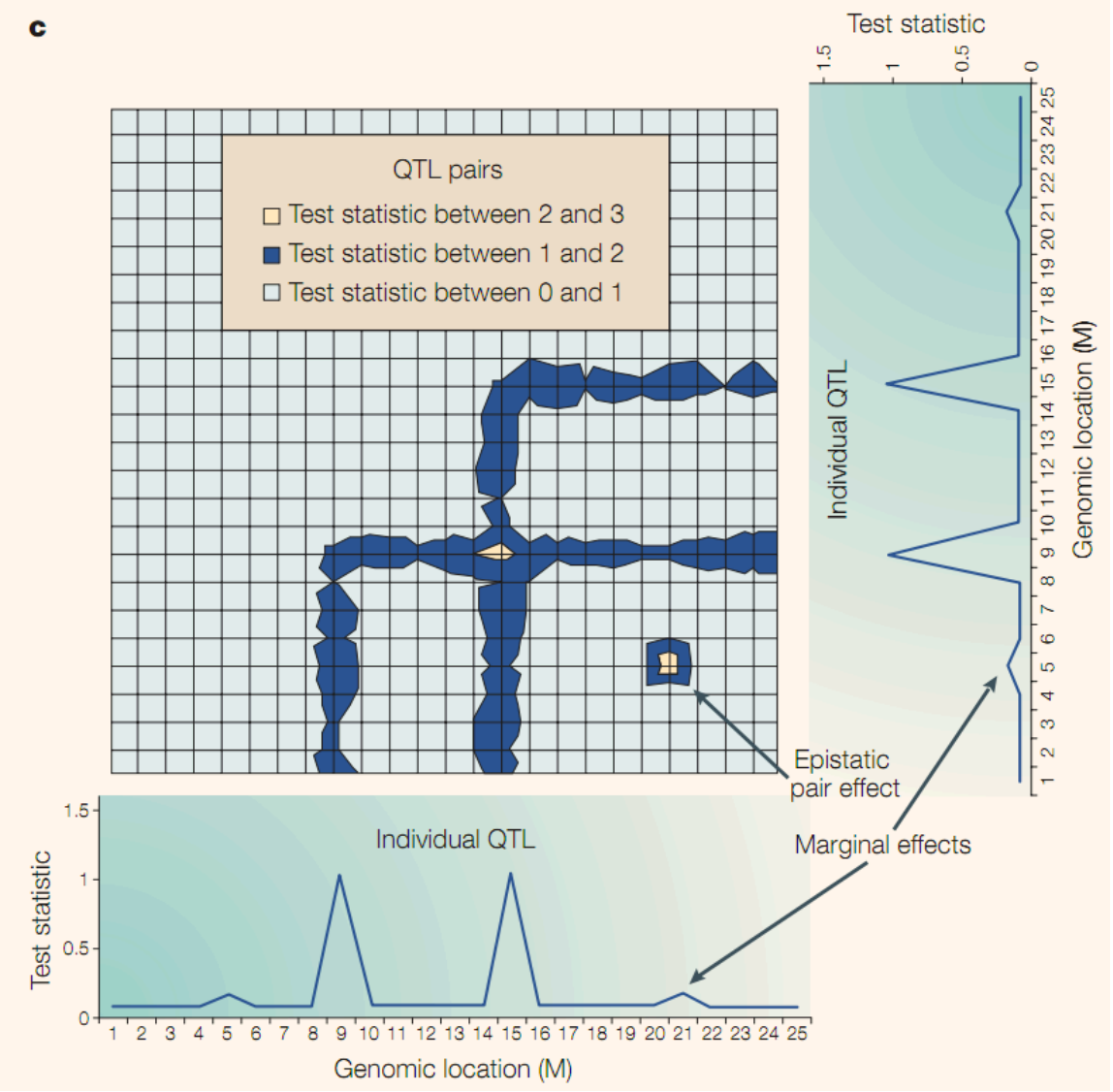


- Co-adaptive epistasis
- Genotypes that are homozygous for alleles of the two loci that originate from the same line (JJ with JJ, or LL with LL) show enhanced performance.
- Almost no marginal effects: average effect of JJ, JL, LL do not differ



- Dominance-by-dominance epistasis
- Double heterozygote (LS, LS) deviates from the phenotype that is expected from the phenotypes of the other heterozygotes.
- Double heterozygotes have a lower phenotype than expected.

Epistatic and Individual QTLs

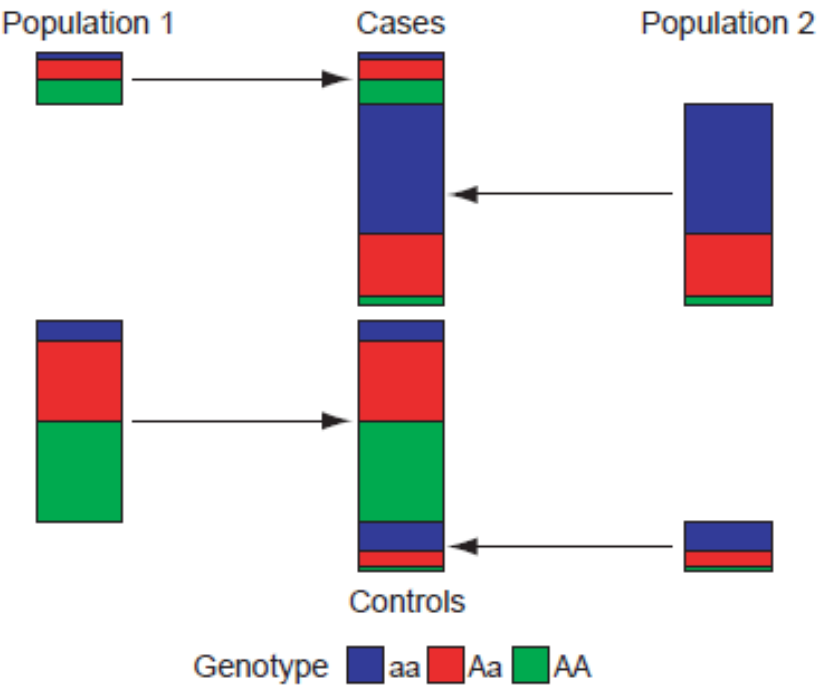


Detecting Epistasis

- Epistatic effects of SNPs can often be detected only if the interacting SNPs are considered jointly
  - The number of candidate SNP interactions is very large
    - For *J* SNPs, *J*x*J* SNP pairs need to be considered for epistasis
    - In general for *J* SNPs and *K*-way interactions, there are $O(J^K)$ candidate interactions
    - Computationally expensive to consider all possible groups of interacting SNPs
    - For a reliable detection of *K*-way interactions, a large sample size is required
  - Multiple testing problem

Population Structure and Association Analysis

- Population structure in data causes false positives
  - Samples in the case population are usually more related
  - Any SNPs more prevalent in the case population will be found significantly associated with the trait.



Accounting for Population Structure in Association Analysis

- Needs to account for population structure in association mapping.

- Careful study design with each population represented in case/control groups in a balanced way.
  - Can be hard to control for population structure during data collection
  - The effect of cryptic population structure

Family-based Design vs. Population-based Design

- Family-based studies
  - The effect of population structure can be controlled by the use of parents' genotypes (e.g., Transmission disequilibrium test (TDT))
  - In practice, collecting genotypes from multiple individuals in a family can be hard. (e.g., late-onset diseases)

- Population-based design
  - Data collection is easier for a large number of unrelated individuals than families.
  - The control samples can be reused in different studies.

Accounting for Population Structure in Association Analysis

- Population-based method
  - Genomic control (Devlin & Roeder, Biometrics 1999)
    - Use the SNPs that are not associated with the trait to remove the effect of population stratification
    - Ignores admixture
  - Structured association (Pritchard et al., AJHG 2000)
    - First run STRUCTURE on genotype data. Within each subpopulation, an association between a genetic marker and the trait is a true association.
  - EigenStrat: principal component analysis (Price et al., Nature Genetics 2006)
    - First run PCA on genotype data to infer the population structure. Perform association analysis after correcting for the population effects in genotype/phenotype data
  - Linear mixed model (Lippert et al., Nature Methods 2011)
    - Model the population effects with random effects