Association Mapping

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BRISTOL

Outline

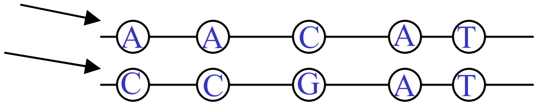
- Definitions / Terminology
- What is (genetic) association?
- How do we test for association?
- When to use association
- HapMap and tagging
- Genome-wide Association
- Sequencing and Rare variants

Definitions

Locus: *Location* on the genome

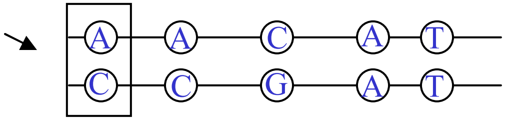
SNP: “Single Nucleotide Polymorphism” a mutation that produces a single base pair change in the DNA sequence

alleles



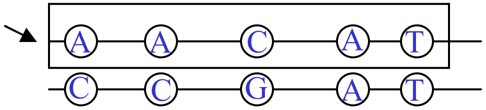
alternate forms of a SNP (mutation)

genotypes



both alleles at a locus form a genotype

haplotypes



the pattern of alleles on a chromosome

QTL: “Quantitative trait locus” a region of the genome that changes the mean value of a quantitative phenotype

What is (genetic) association?

Correlation between an allele/genotype/haplotype and a trait of interest

Genetic Association
Three Common Forms

- Direct Association
  - Mutant or ‘susceptible’ polymorphism
  - Allele of interest is itself involved in phenotype
  - ~70% of Cystic Fibrosis patients have a deletion of 3 base pairs resulting in the loss of a phenylalanine amino acid at position 508 of the *CFTR* gene

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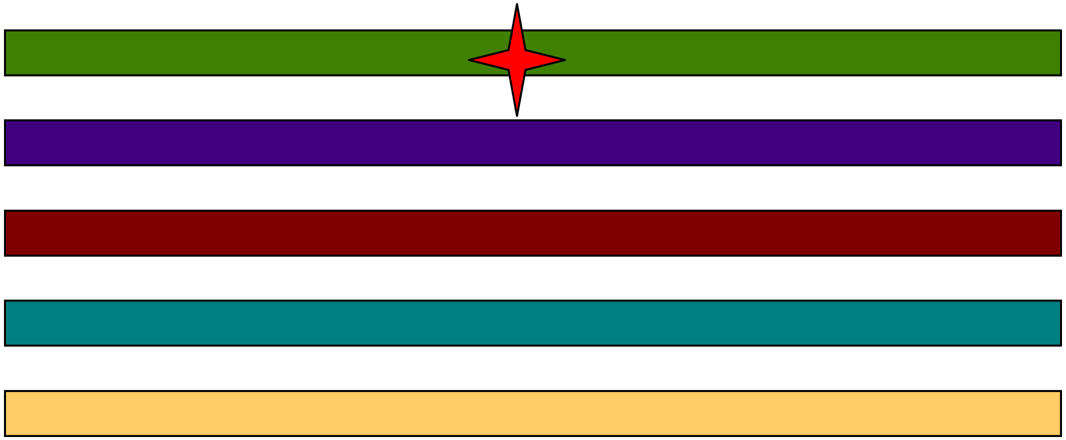
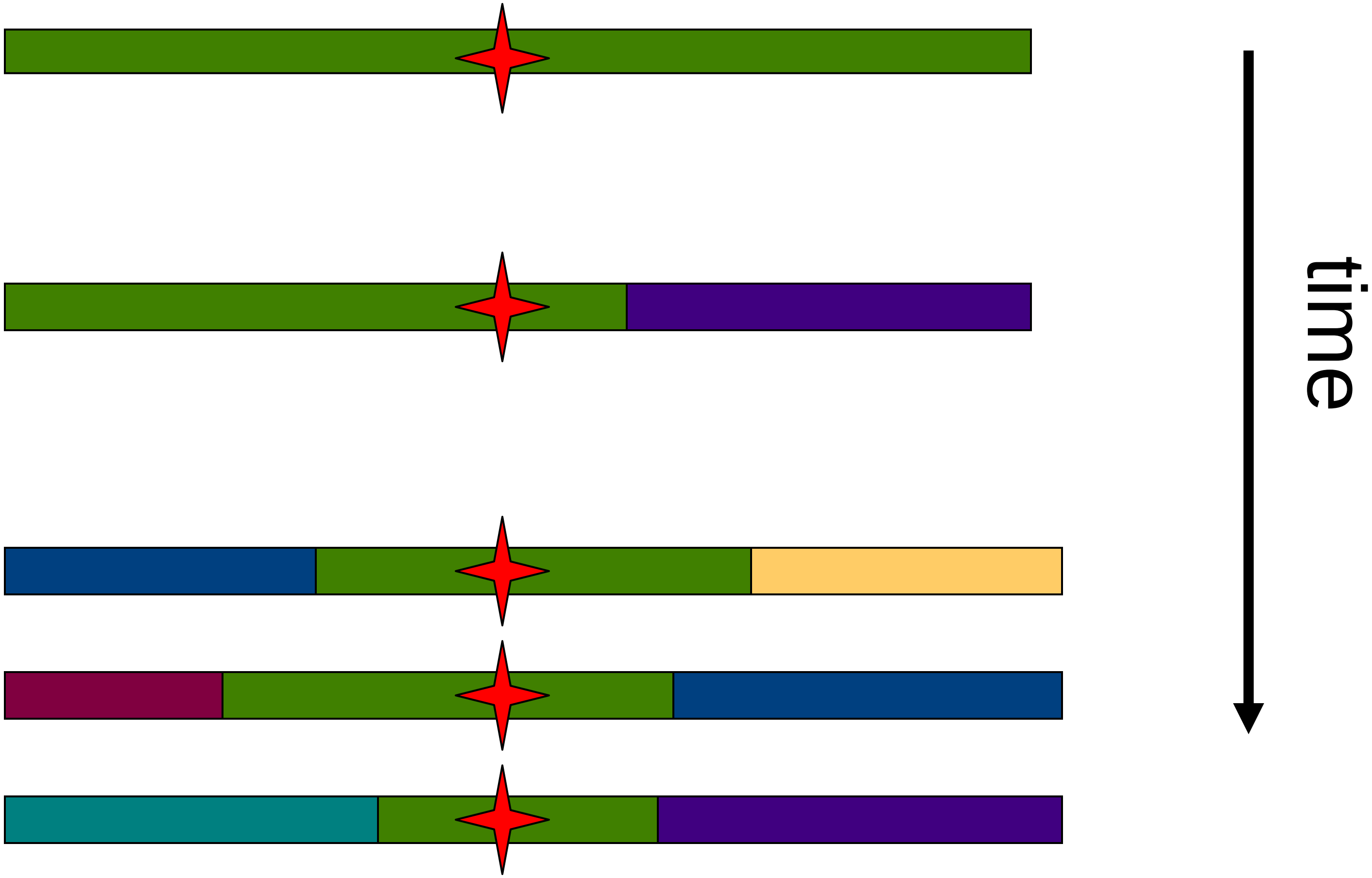
- Indirect Association
  - Allele itself is not involved, but a nearby correlated variant changes phenotype

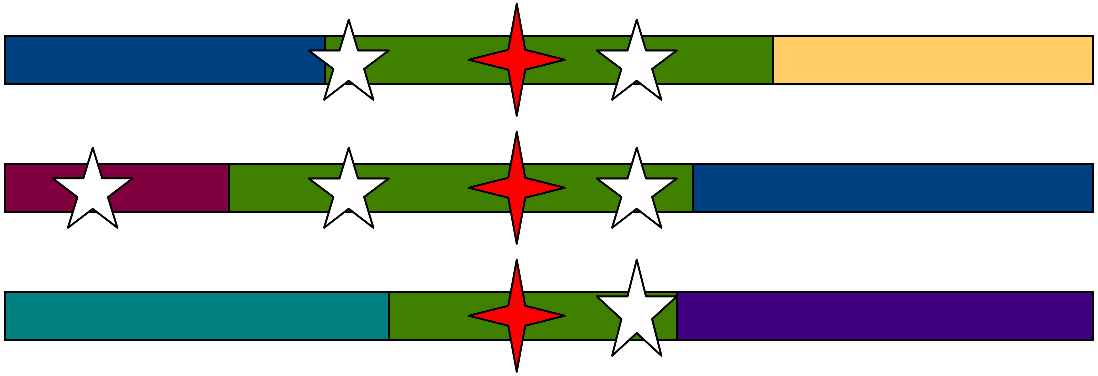
Indirect association and Linkage disequilibrium



Indirect association and Linkage disequilibrium



Linkage Disequilibrium



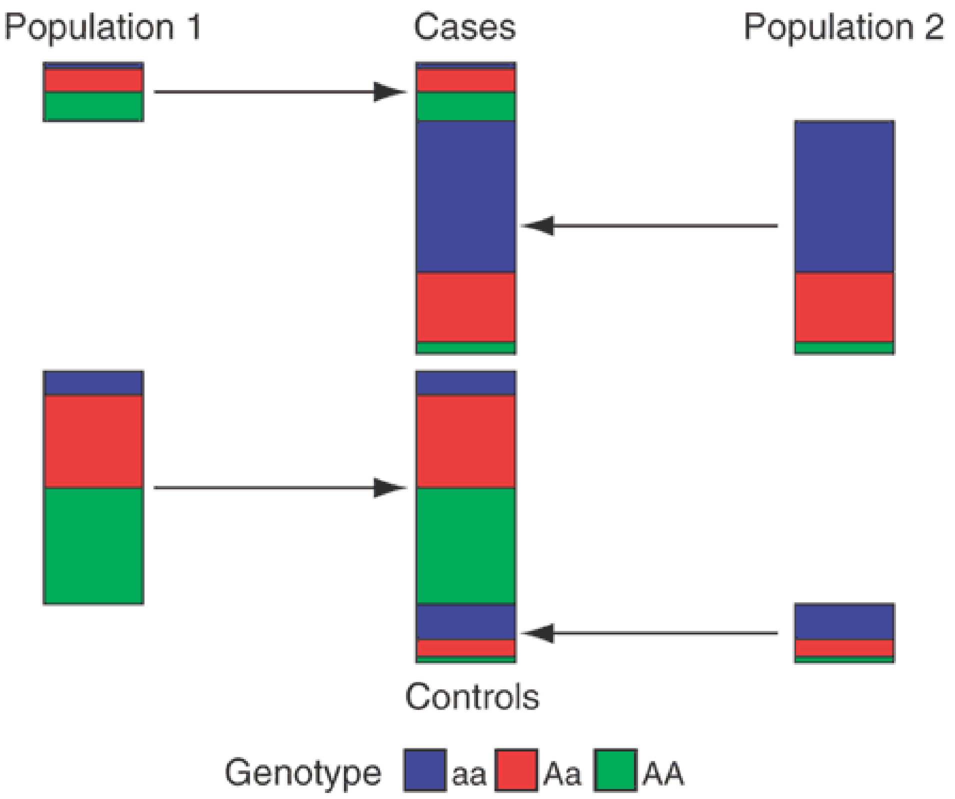
Linkage disequilibrium means that we don’t need to genotype the exact aetiological variant, but only a variant that is correlated with it

Genetic Association

Three Common Forms

- Direct Association
  - Mutant or ‘susceptible’ polymorphism
  - Allele of interest is itself involved in phenotype
- Indirect Association
  - Allele itself is not involved, but a nearby correlated marker changes phenotype
- Spurious association
  - Apparent association not related to genetic aetiology (e.g. population stratification)

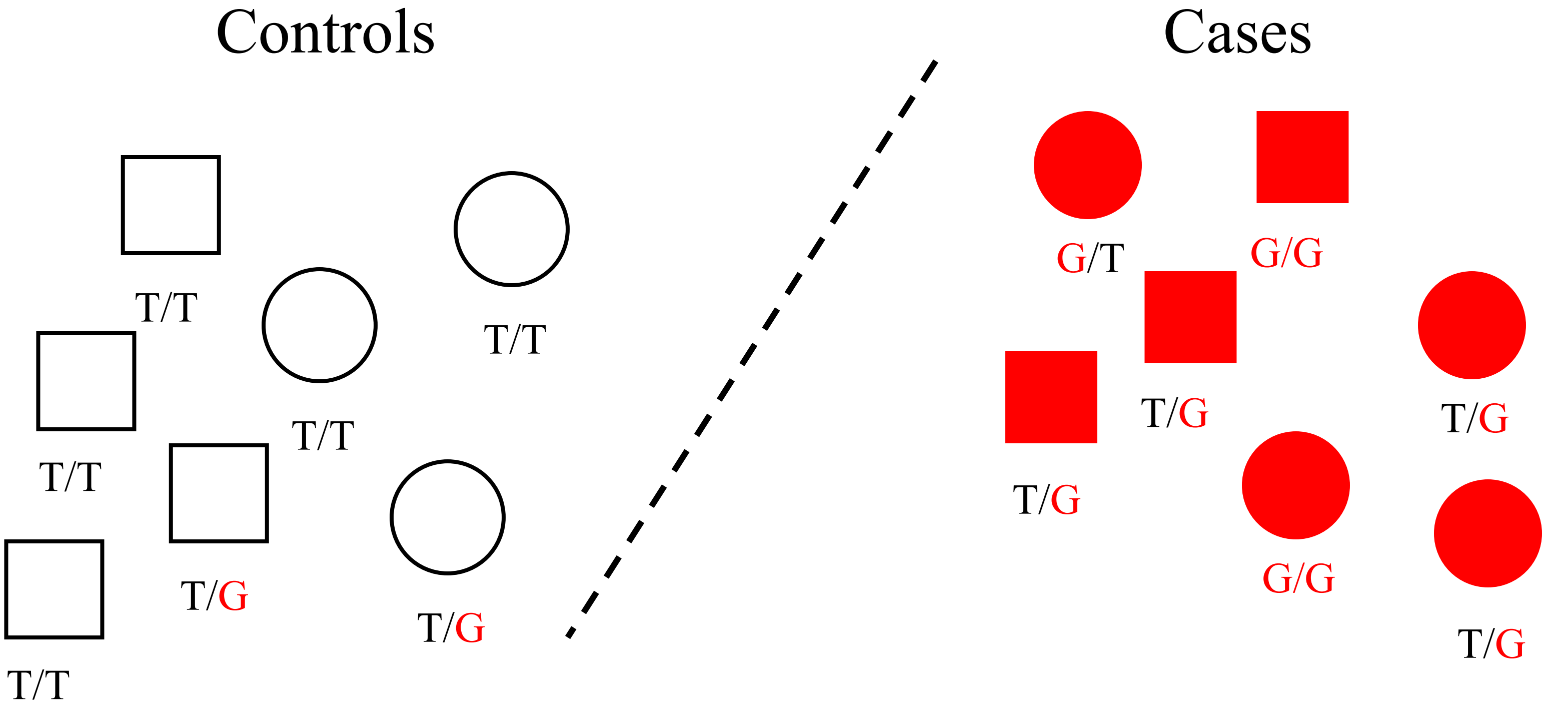
Population Stratification



Marchini, *Nat Genet.* 2004

How do we test for association?

Genetic Case Control Study



Allele G is ‘associated’ with disease

Allele-based tests

- Each individual contributes two counts to 2x2 table.
- Test of association

$$X^2 = \sum_{i=0,1} \sum_{j=A,U} \frac{\left(n_{ij} - E\left[n_{ij}\right]\right)^2}{E\left[n_{ij}\right]}$$

where

$$E\left[n_{ij}\right] = \frac{n_{i\cdot} n_{\cdot j}}{n_{\cdot\cdot}}$$

- X^2 has χ^2 distribution with 1 degrees of freedom under null hypothesis.

	Cases	Controls	Total
G	n_{1A}	n_{1U}	$n_{1\cdot}$
T	n_{0A}	n_{0U}	$n_{0\cdot}$
Total	$n_{\cdot A}$	$n_{\cdot U}$	$n_{\cdot\cdot}$

Genotypic tests

- SNP marker data can be represented in 2x3 table.
- Test of association

$$X^2 = \sum_{i=0,1,2} \sum_{j=A,U} \frac{\left(n_{ij} - E\left[n_{ij}\right]\right)^2}{E\left[n_{ij}\right]}$$

where

$$E\left[n_{ij}\right] = \frac{n_{i\cdot} n_{\cdot j}}{n_{\cdot\cdot}}$$

- X^2 has χ^2 distribution with 2 degrees of freedom under null hypothesis.

	Cases	Controls	Total
GG	n_{2A}	n_{2U}	$n_{2\cdot}$
GT	n_{1A}	n_{1U}	$n_{1\cdot}$
TT	n_{0A}	n_{0U}	$n_{0\cdot}$
Total	$n_{\cdot A}$	$n_{\cdot U}$	$n_{\cdot\cdot}$

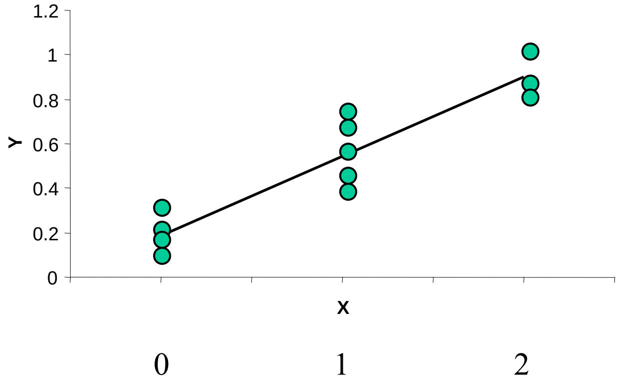
Simple Regression Model of Association (Unrelated individuals)

$$Y_i = \alpha + \beta X_i + e_i$$

where

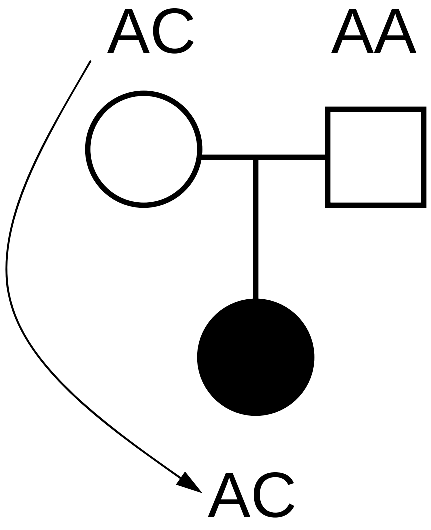
$Y_i =$ trait value for individual i

$X_i =$ number of ‘A’ alleles an individual has



Association test is whether $\beta > 0$

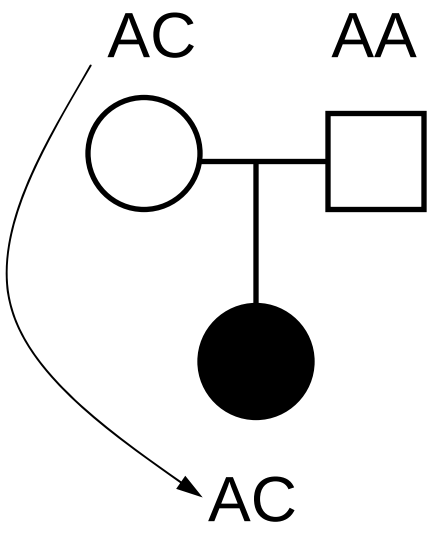
Transmission Disequilibrium Test



•Rationale: Related individuals have to be from the same population

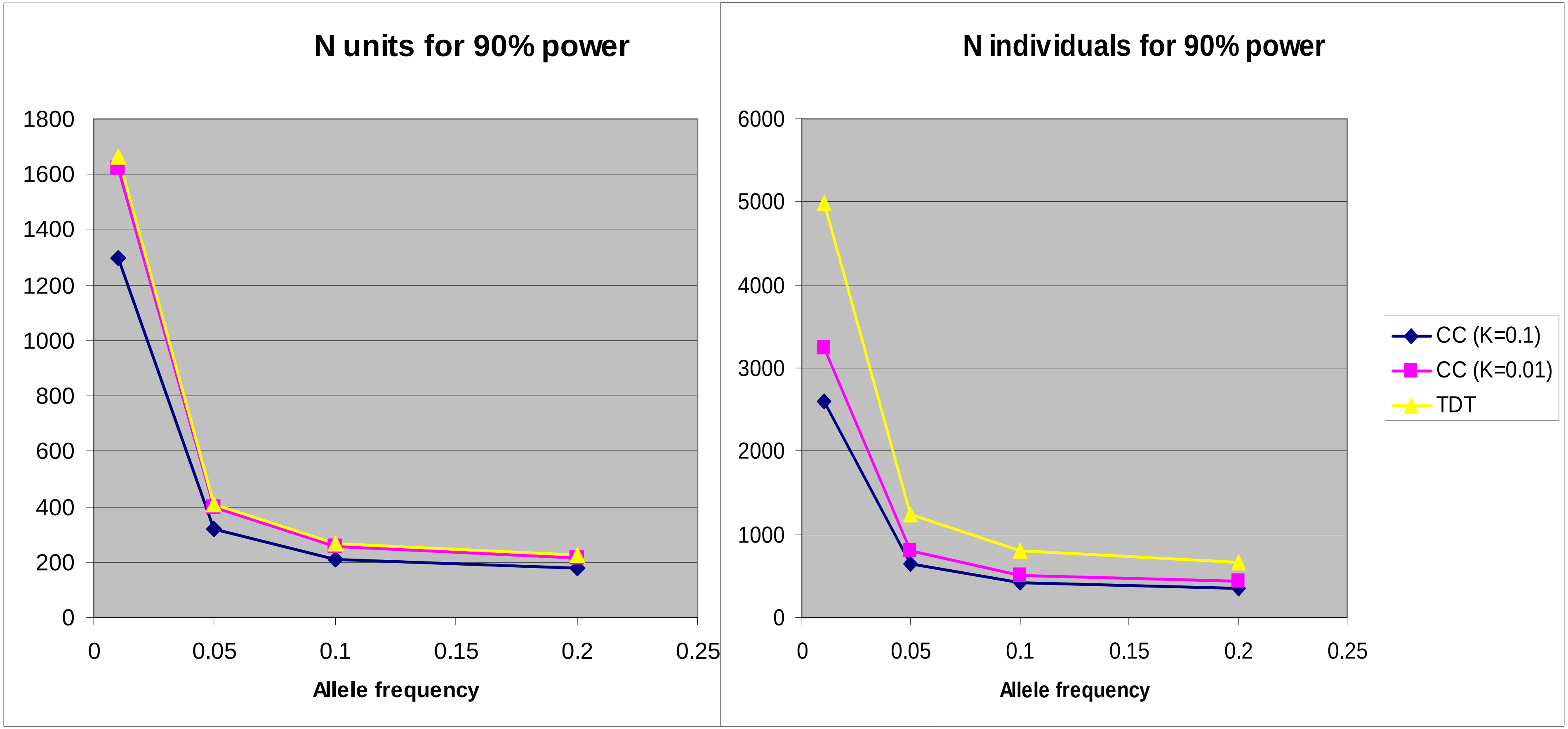
•Compare number of times heterozygous parents transmit “A” vs “C” allele to affected offspring

Transmission Disequilibrium Test

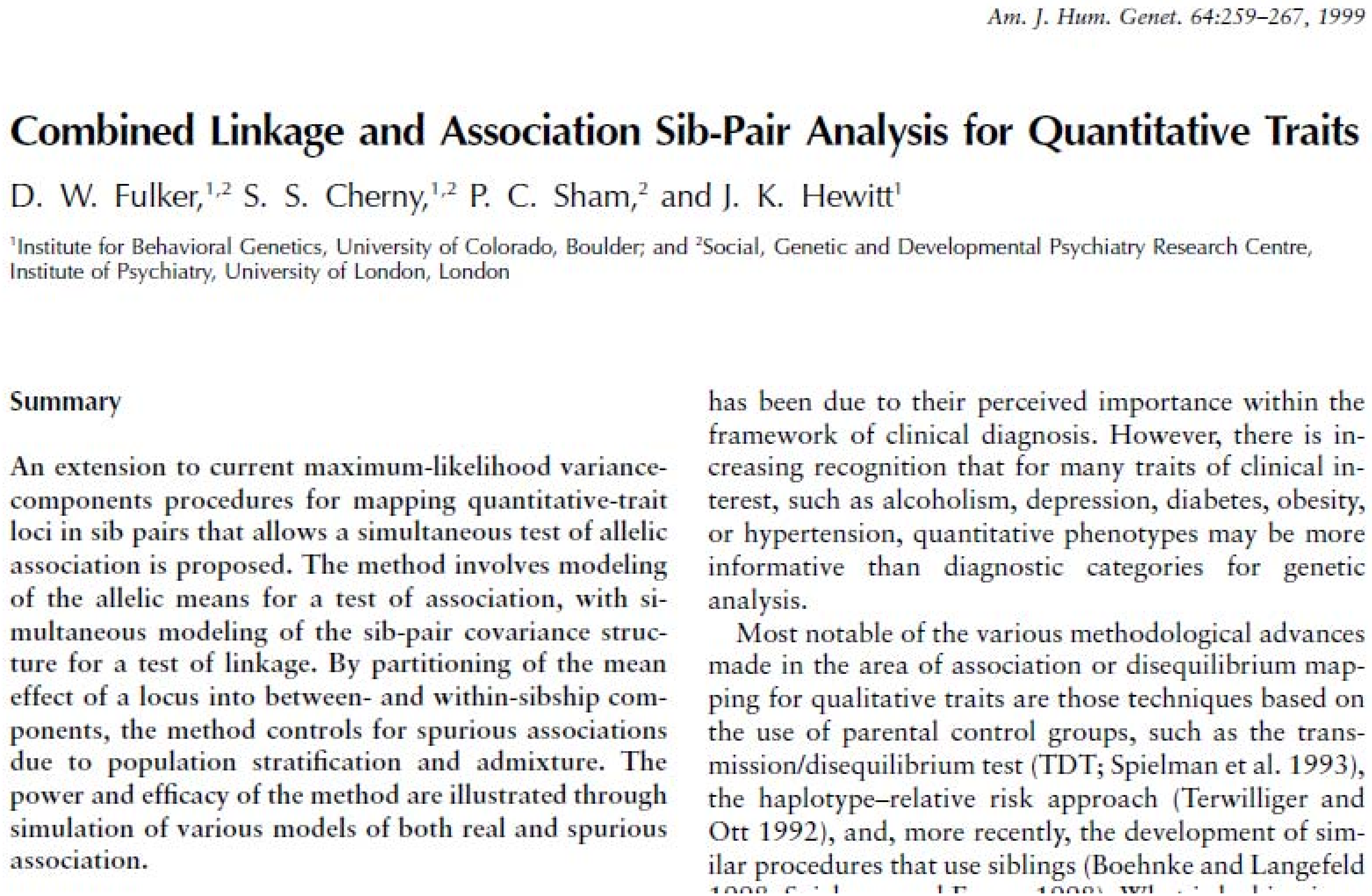


- Difficult to gather families
- Difficult to get parents for late onset / psychiatric conditions
- Inefficient for genotyping (particularly GWA)

Case-control versus TDT



p = 0.1; RAA = RAa = 2



Am. J. Hum. Genet. 64:259–267, 1999

Combined Linkage and Association Sib-Pair Analysis for Quantitative Traits

D. W. Fulker,[1,2] S. S. Cherny,[1,2] P. C. Sham,[2] and J. K. Hewitt[1]

[1]Institute for Behavioral Genetics, University of Colorado, Boulder; and [2]Social, Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry, University of London, London

Summary

An extension to current maximum-likelihood variance-components procedures for mapping quantitative-trait loci in sib pairs that allows a simultaneous test of allelic association is proposed. The method involves modeling of the allelic means for a test of association, with simultaneous modeling of the sib-pair covariance structure for a test of linkage. By partitioning of the mean effect of a locus into between- and within-sibship components, the method controls for spurious associations due to population stratification and admixture. The power and efficacy of the method are illustrated through simulation of various models of both real and spurious association.

has been due to their perceived importance within the framework of clinical diagnosis. However, there is increasing recognition that for many traits of clinical interest, such as alcoholism, depression, diabetes, obesity, or hypertension, quantitative phenotypes may be more informative than diagnostic categories for genetic analysis.

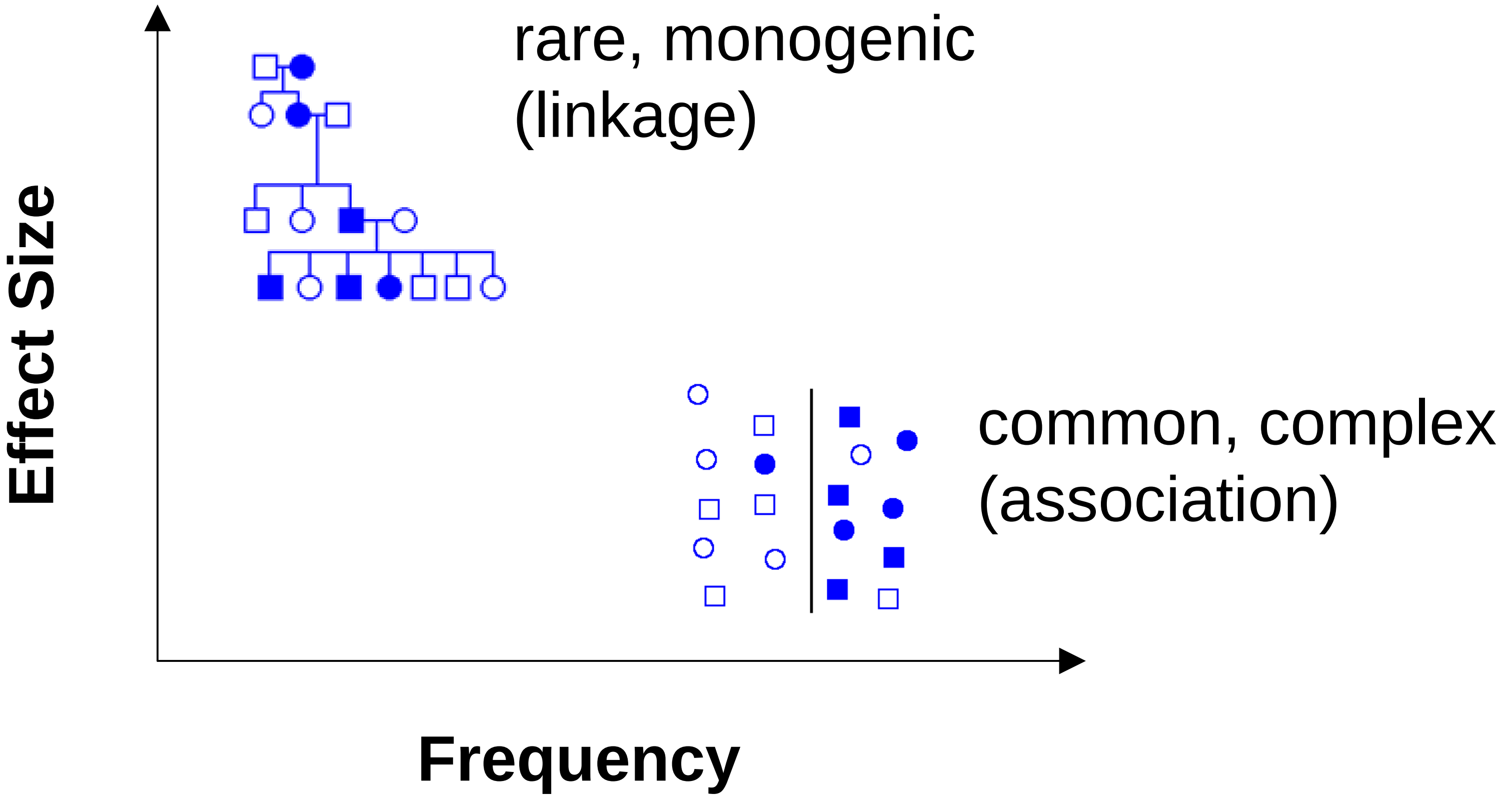
Most notable of the various methodological advances made in the area of association or disequilibrium mapping for qualitative traits are those techniques based on the use of parental control groups, such as the transmission/disequilibrium test (TDT; Spielman et al. 1993), the haplotype–relative risk approach (Terwilliger and Ott 1992), and, more recently, the development of similar procedures that use siblings (Boehnke and Langefeld

$$\hat{y}_{ij} = \mu + \beta_a g_{ij} = \mu + \beta_b b_i + \beta_w w_{ij}$$

$$\Sigma_i = \begin{pmatrix} \sigma_q^2 + \sigma_c^2 + \sigma_e^2 & \hat{\pi}_i \sigma_q^2 + \sigma_c^2 \\ \hat{\pi}_i \sigma_q^2 + \sigma_c^2 & \sigma_q^2 + \sigma_c^2 + \sigma_e^2 \end{pmatrix}$$

When to use association...

Methods of gene hunting



rare, monogenic
(linkage)

common, complex
(association)

Effect Size

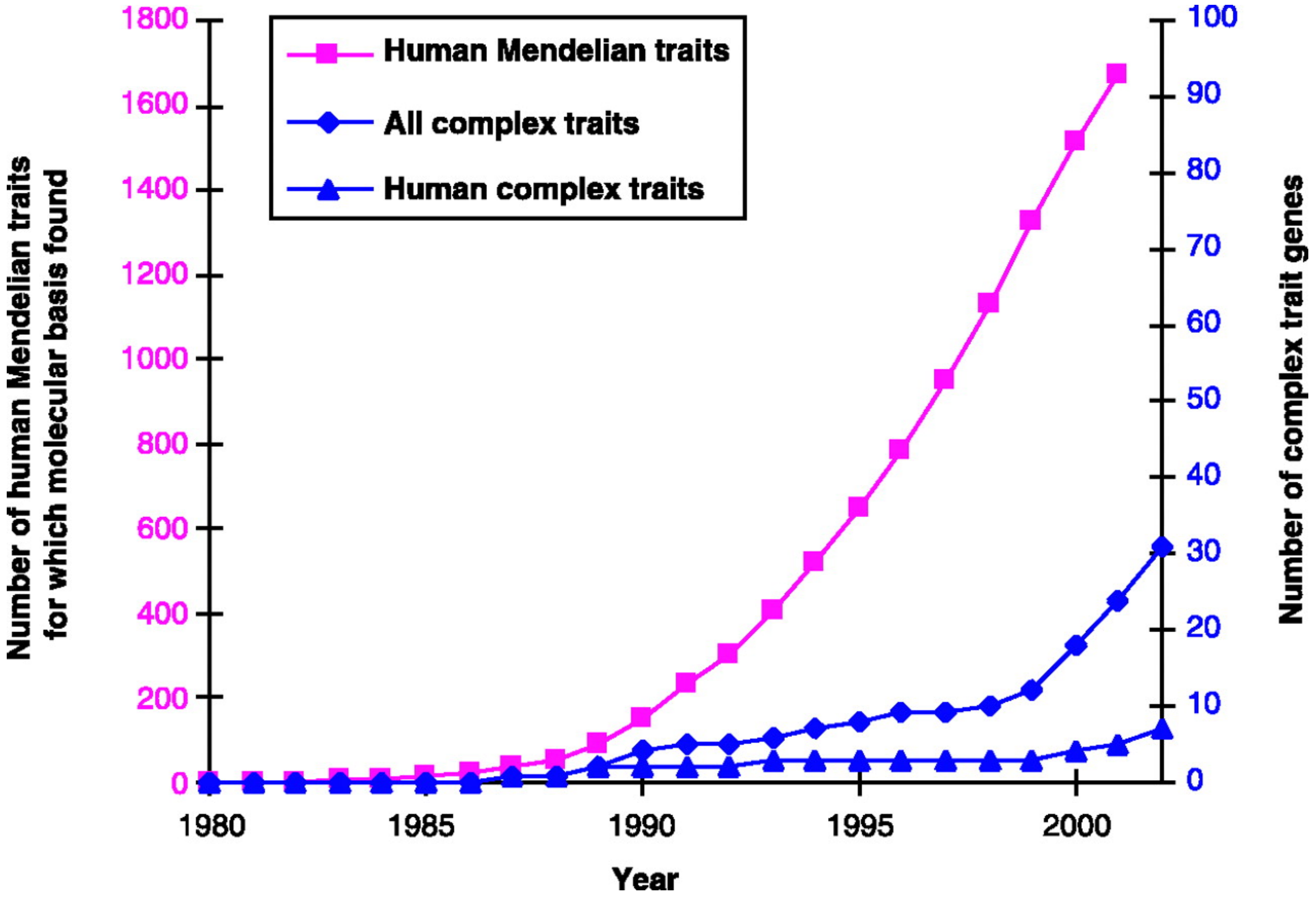
Frequency

Association Summary

1. Families or unrelateds
2. Matching/ethnicity crucial
3. Many markers req for genome coverage (10^5 – 10^6 SNPs)
4. Powerful design
5. Ok for initial detection; good for fine-mapping
6. Powerful for common variants; rare variants difficult

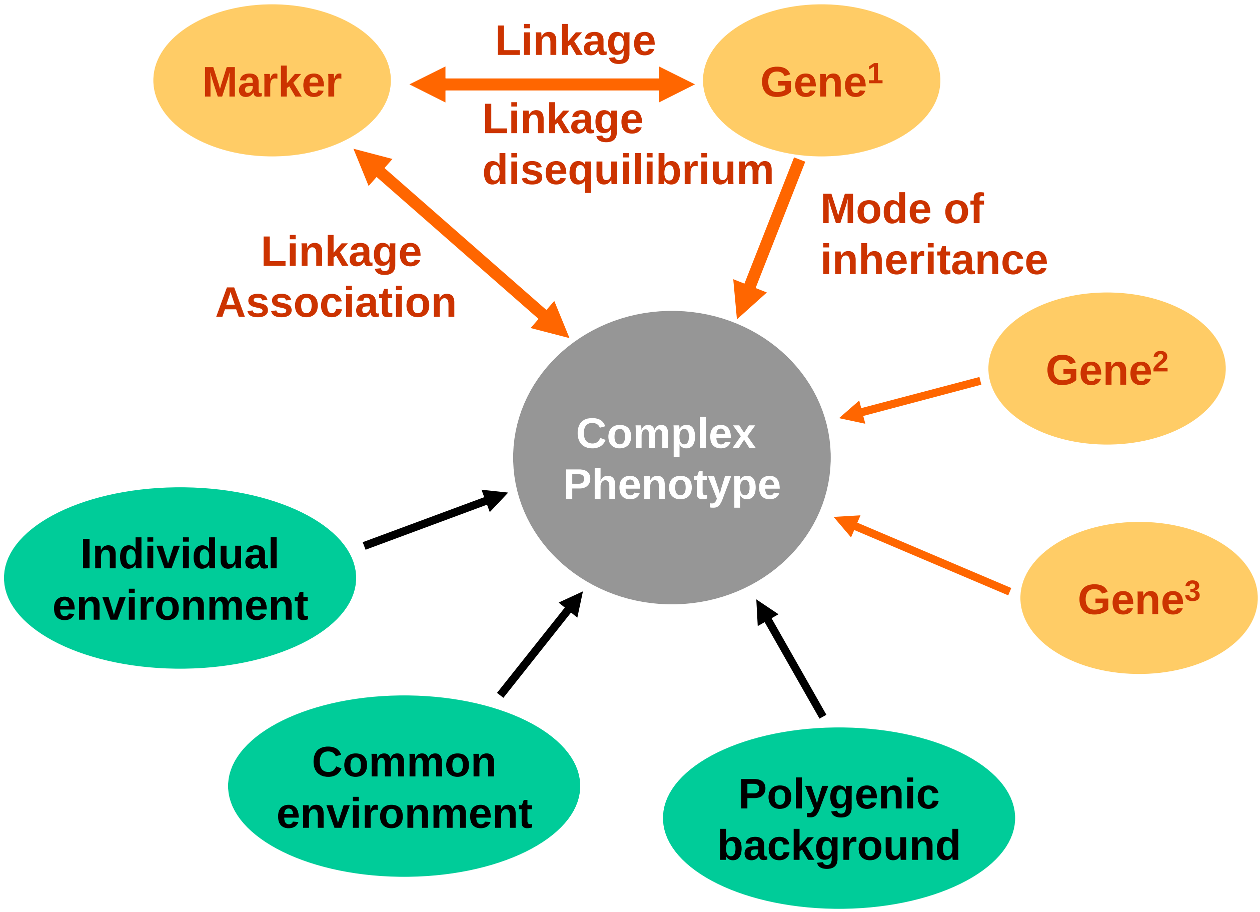
HapMap and Tagging

Historical gene mapping



Glazier et al, *Science* (2002).

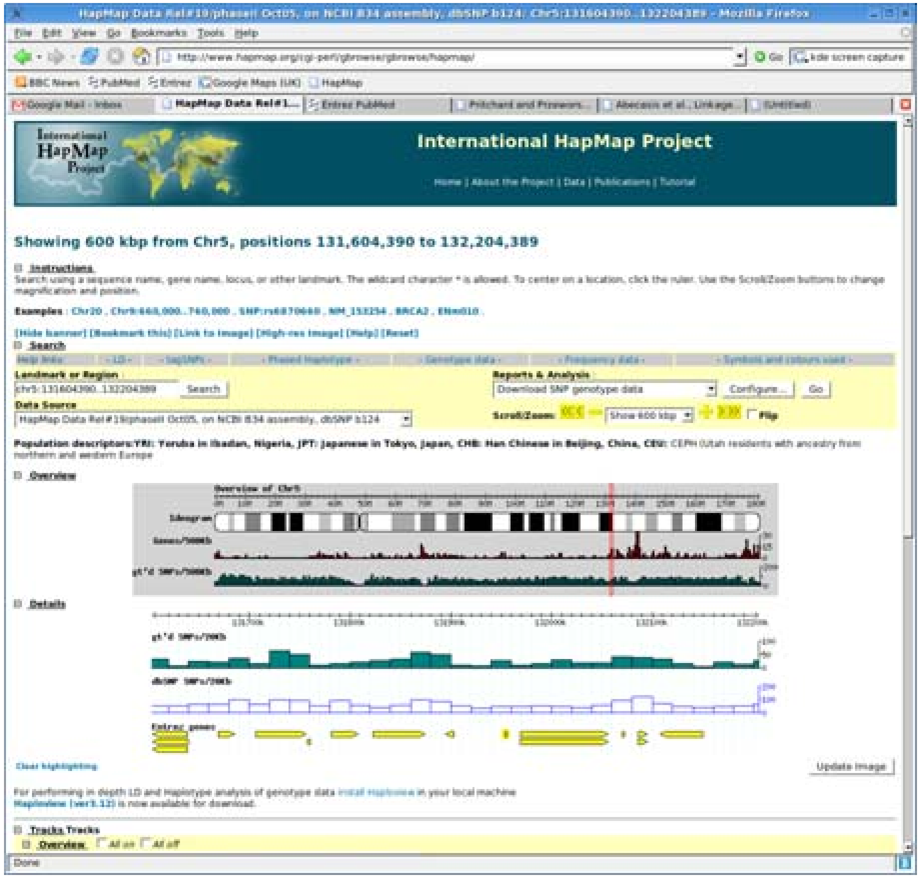
Reasons for Failure?



- Inadequate Marker Coverage (Candidate gene studies)

Weiss & Terwilliger (2000) *Nat Genet*

Enabling association studies: HapMap



HapMap Data Rel#19/phaseII Oct05, on NCBI B34 assembly, dbSNP b124: Chr5:131604390..132204389 - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.hapmap.org/cgi-perl/gbrowse/gbrowse/hapmap/
Go
kde screen capture
BBC News
PubMed
Entrez
Google Maps (UK)
HapMap
Google Mail - Inbox
HapMap Data Rel#1...
Entrez PubMed
Pritchard and Przeworsk...
Abecasis et al., Linkage...
(Untitled)
International HapMap Project
International HapMap Project
Home | About the Project | Data | Publications | Tutorial
Overview of Chr5
Ideogram
Genes/500kb
gt'd SNPs/500kb
Details
gt'd SNPs/20kb
dbSNP SNPs/20kb
Entrez genes
Clear highlighting
Update Image
Done

Showing 600 kbp from Chr5, positions 131,604,390 to 132,204,389

⊟ **Instructions**

Search using a sequence name, gene name, locus, or other landmark. The wildcard character * is allowed. To center on a location, click the ruler. Use the Scroll/Zoom buttons to change magnification and position.

Examples : Chr20 , Chr9:660,000..760,000 , SNP:rs6870660 , NM_153254 , BRCA2 , ENm010 .

[Hide banner] [Bookmark this] [Link to image] [High-res image] [Help] [Reset]

⊟ **Search**

Help links: - LD - - tagSNPs - - Phased haplotype - - Genotype data - - Frequency data - - Symbols and colours used -

Landmark or Region : chr5:131604390..132204389 Search

Reports & Analysis : Download SNP genotype data Configure... Go

Data Source : HapMap Data Rel#19/phaseII Oct05, on NCBI B34 assembly, dbSNP b124

Scroll/Zoom: Show 600 kbp □ Flip

Population descriptors:YRI: Yoruba in Ibadan, Nigeria, JPT: Japanese in Tokyo, Japan, CHB: Han Chinese in Beijing, China, CEU: CEPH (Utah residents with ancestry from northern and western Europe

⊟ **Overview**

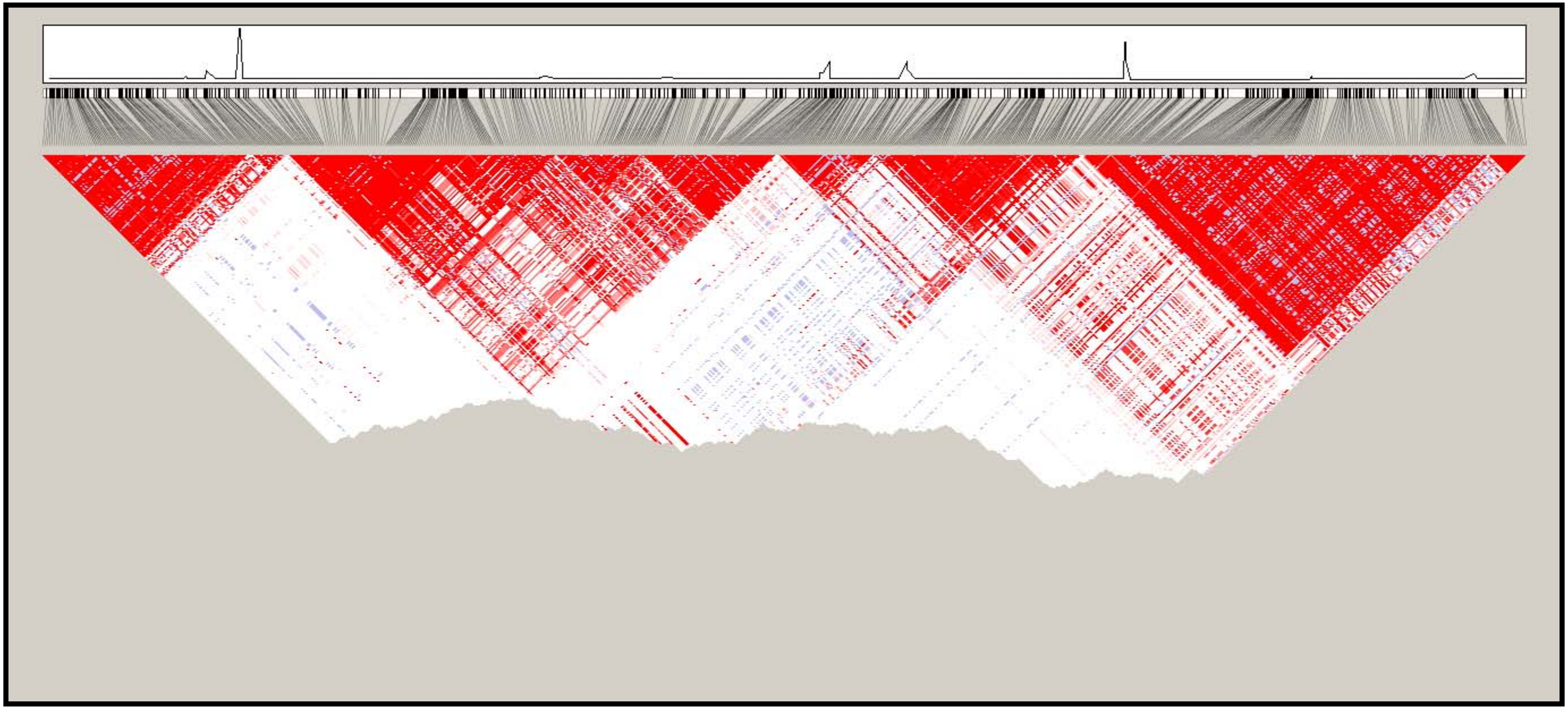
⊟ **Details**

For performing in depth LD and Haplotype analysis of genotype data install Haploview in your local machine
Haploview (ver3.12) is now available for download.

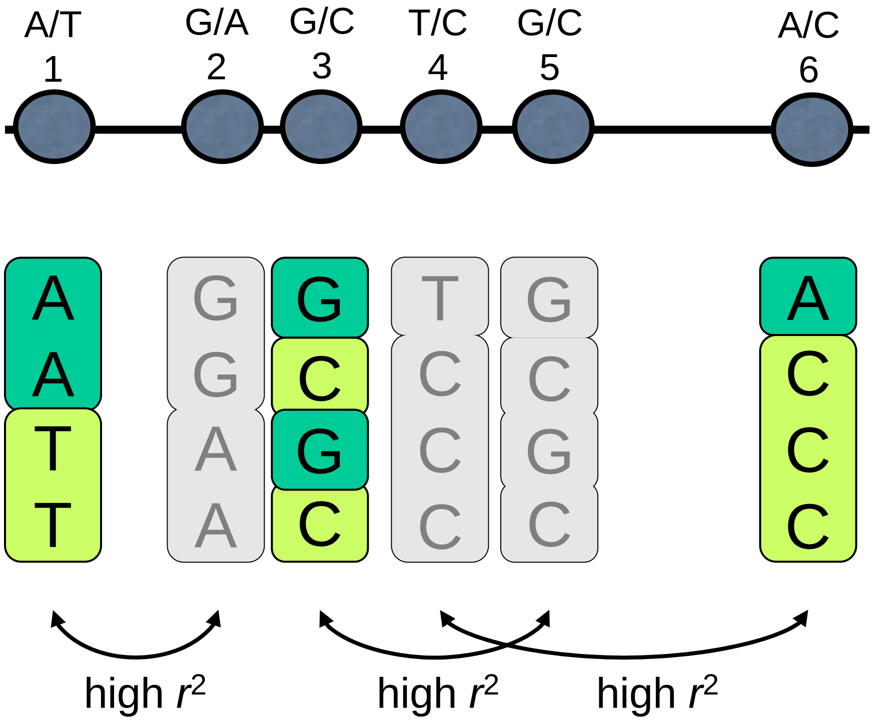
⊟ **Tracks Tracks**

⊟ **Overview** □ All on □ All off

Visualizing empirical LD



Pairwise tagging



Tags:

SNP 1
SNP 3
SNP 6

3 in total

Test for association:

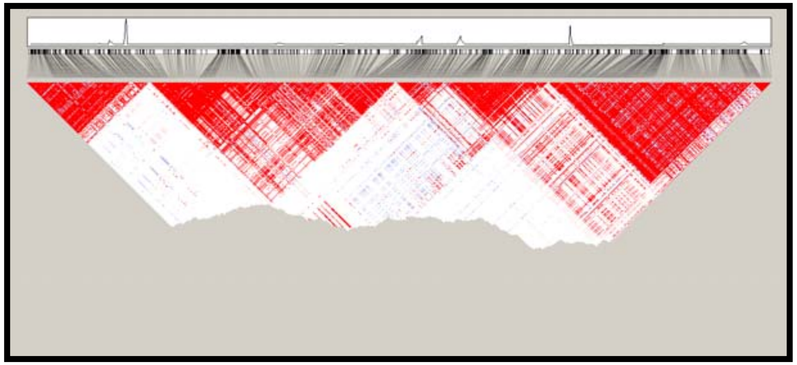
SNP 1
SNP 3
SNP 6

Carlson *et al.* (2004) *AJHG* **74**:106

Genome-wide Association

Enabling Genome-wide Association Studies

- HAPlotype MAP



- High throughput genotyping

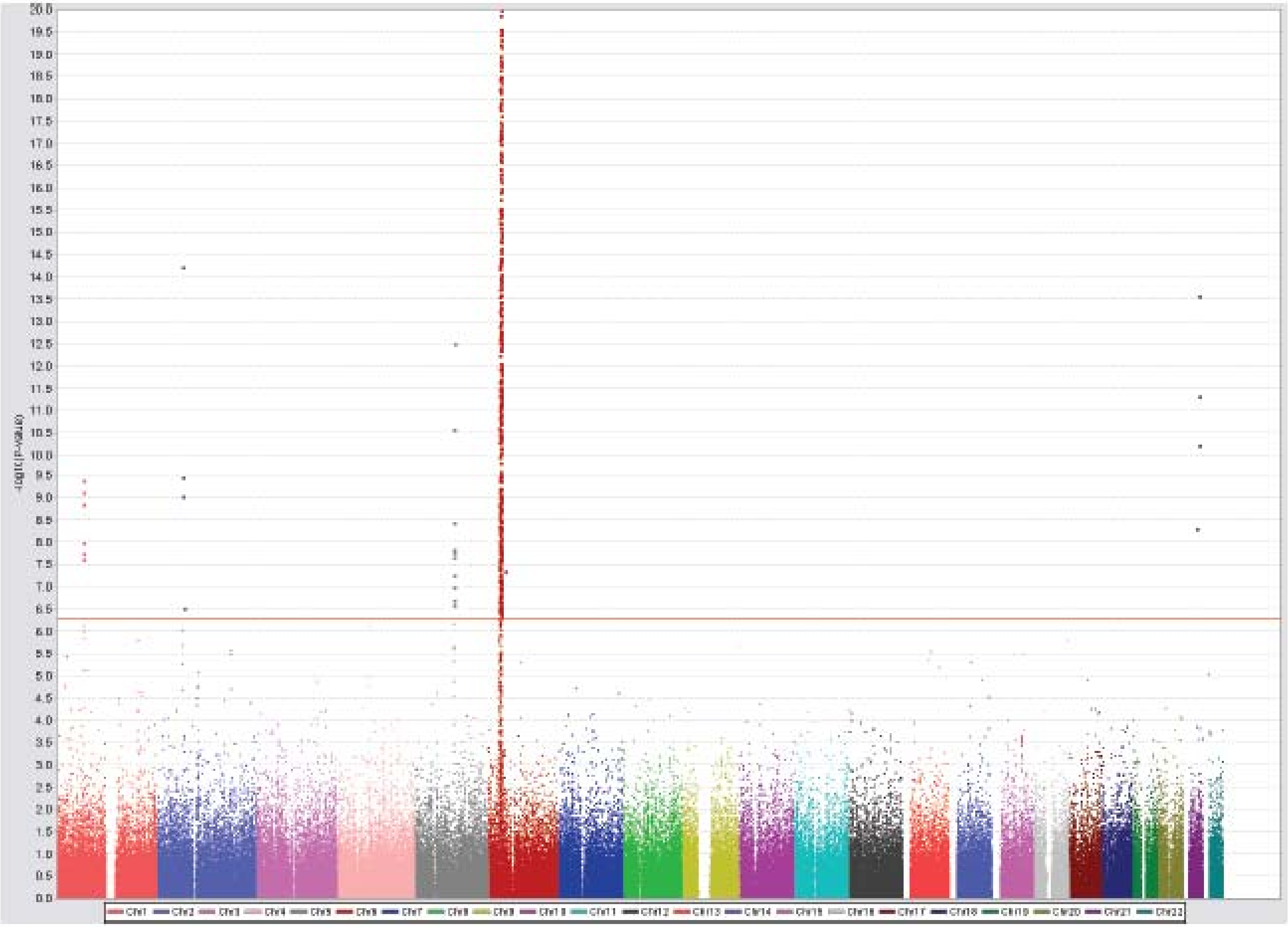
- Large cohorts

AUSTRALIAN twin REGISTRY

VRIJE UNIVERSITEIT NEDERLANDS TWEELINGEN REGISTER

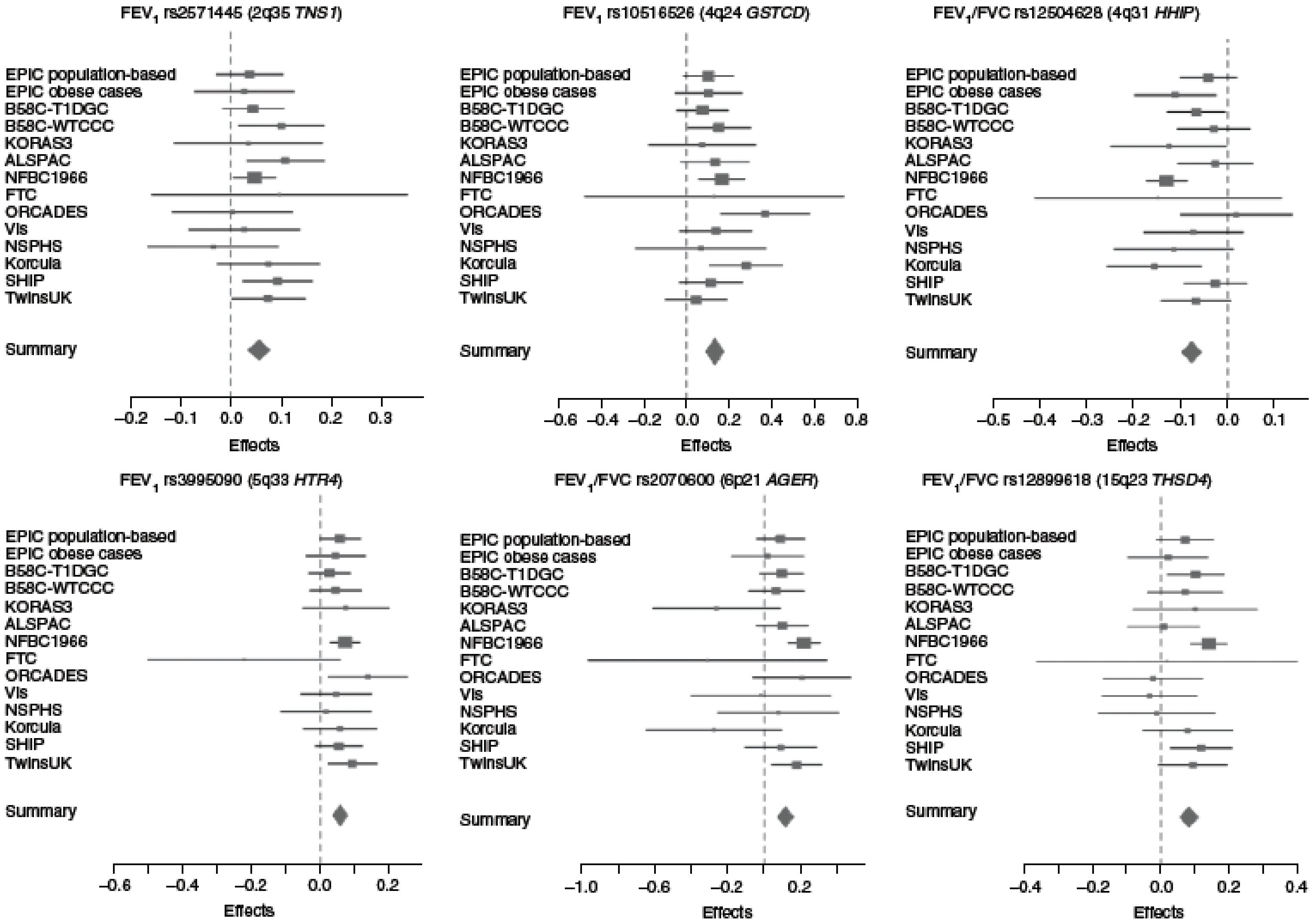
CHILDREN OF THE 90s

Genome-wide Association Studies



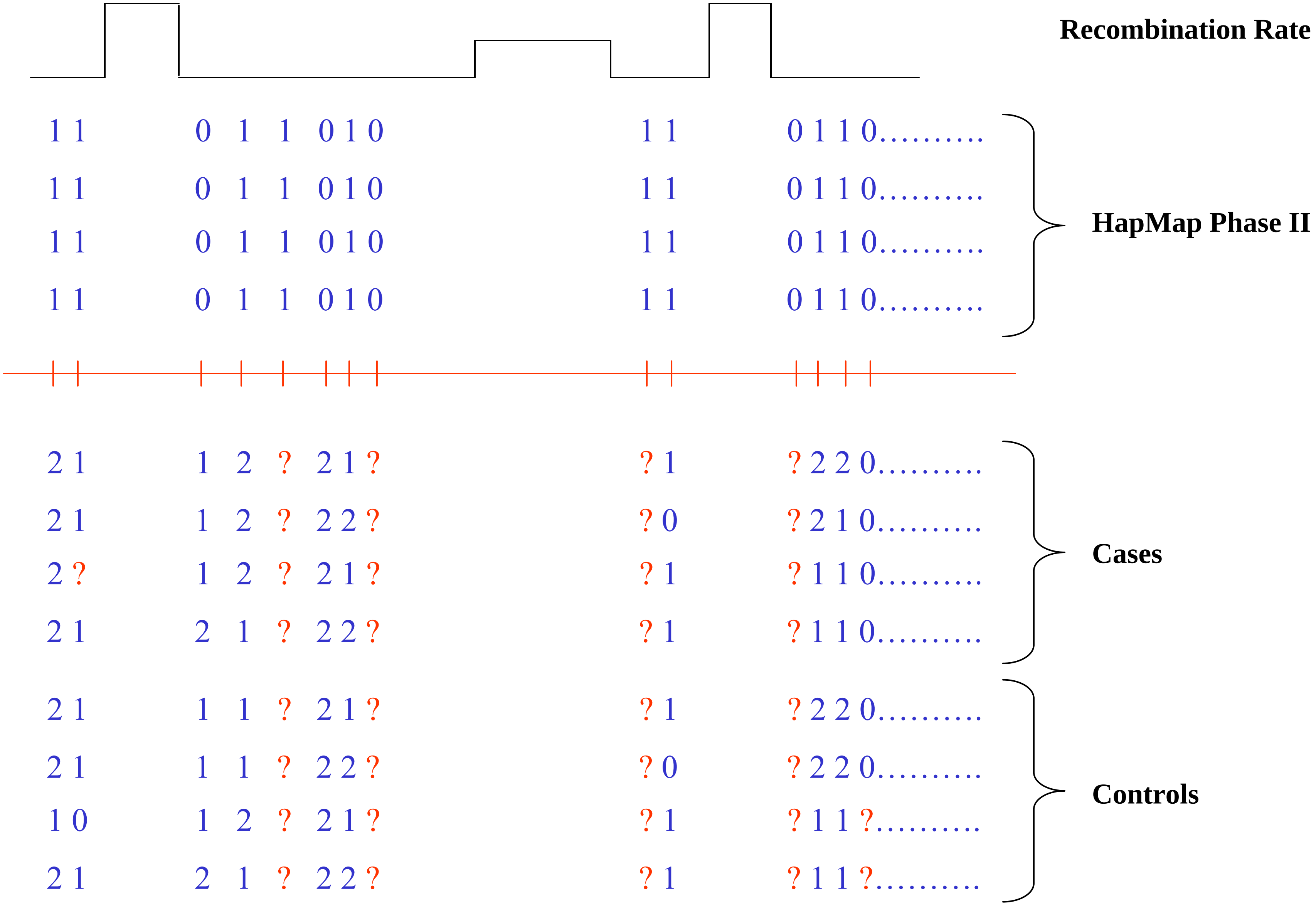
The Australo-Anglo-American Ankylosing Spondylitis Consortium (2010) *Nature Genetics*

Meta-analysis



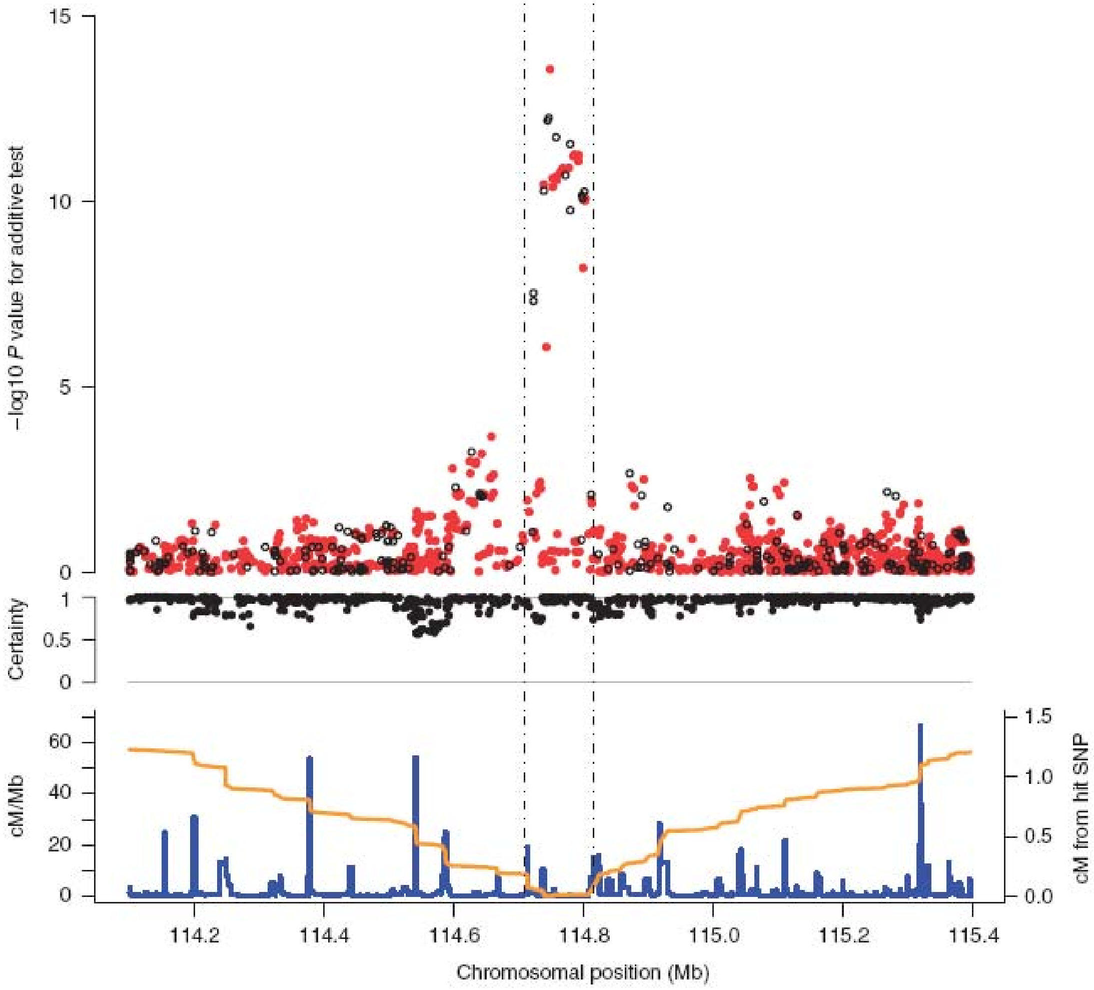
Repapi et al. (2009) *Nature Genetics*

Imputation



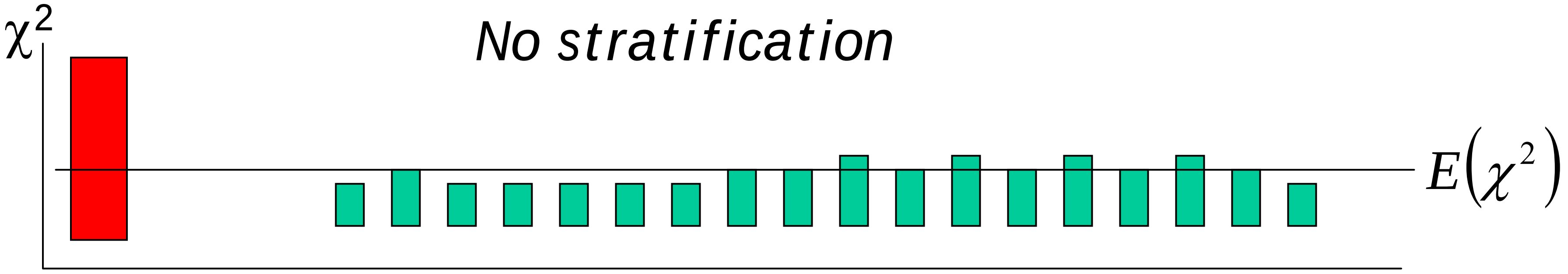
1	1	0	1	1	0	1	0	1	1	0	1	1	0	....	HapMap Phase II
1	1	0	1	1	0	1	0	1	1	0	1	1	0	....	
1	1	0	1	1	0	1	0	1	1	0	1	1	0	....	
1	1	0	1	1	0	1	0	1	1	0	1	1	0	....	
2	1	1	2	?	2	1	?	?	1	?	2	2	0	....	Cases
2	1	1	2	?	2	2	?	?	0	?	2	1	0	....	
2	?	1	2	?	2	1	?	?	1	?	1	1	0	....	
2	1	2	1	?	2	2	?	?	1	?	1	1	0	....	
2	1	1	1	?	2	1	?	?	1	?	2	2	0	....	Controls
2	1	1	1	?	2	2	?	?	0	?	2	2	0	....	
1	0	1	2	?	2	1	?	?	1	?	1	1	?	....	
2	1	2	1	?	2	2	?	?	1	?	1	1	?	....	

Imputation



15
10
5
0
−log10 P value for additive test
1
0.5
0
Certainty
60
40
20
0
cM/Mb
1.5
1.0
0.5
0.0
cM from hit SNP
114.2
114.4
114.6
114.8
115.0
115.2
115.4
Chromosomal position (Mb)

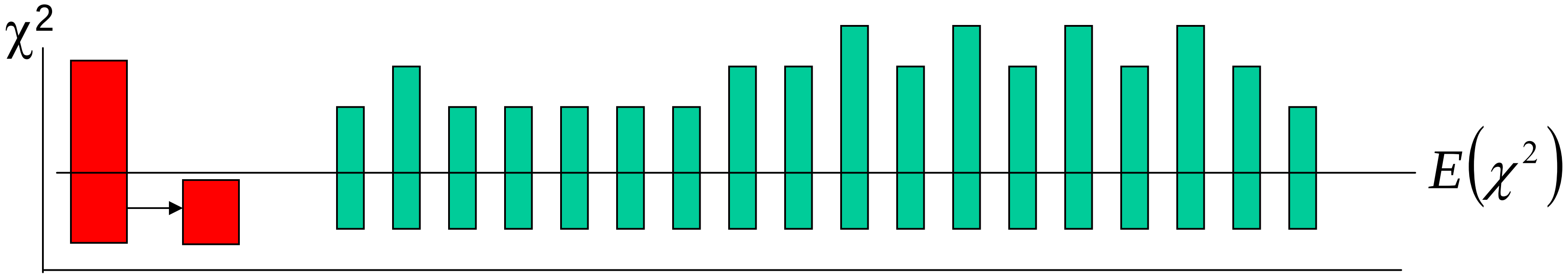
Genomic control



No stratification

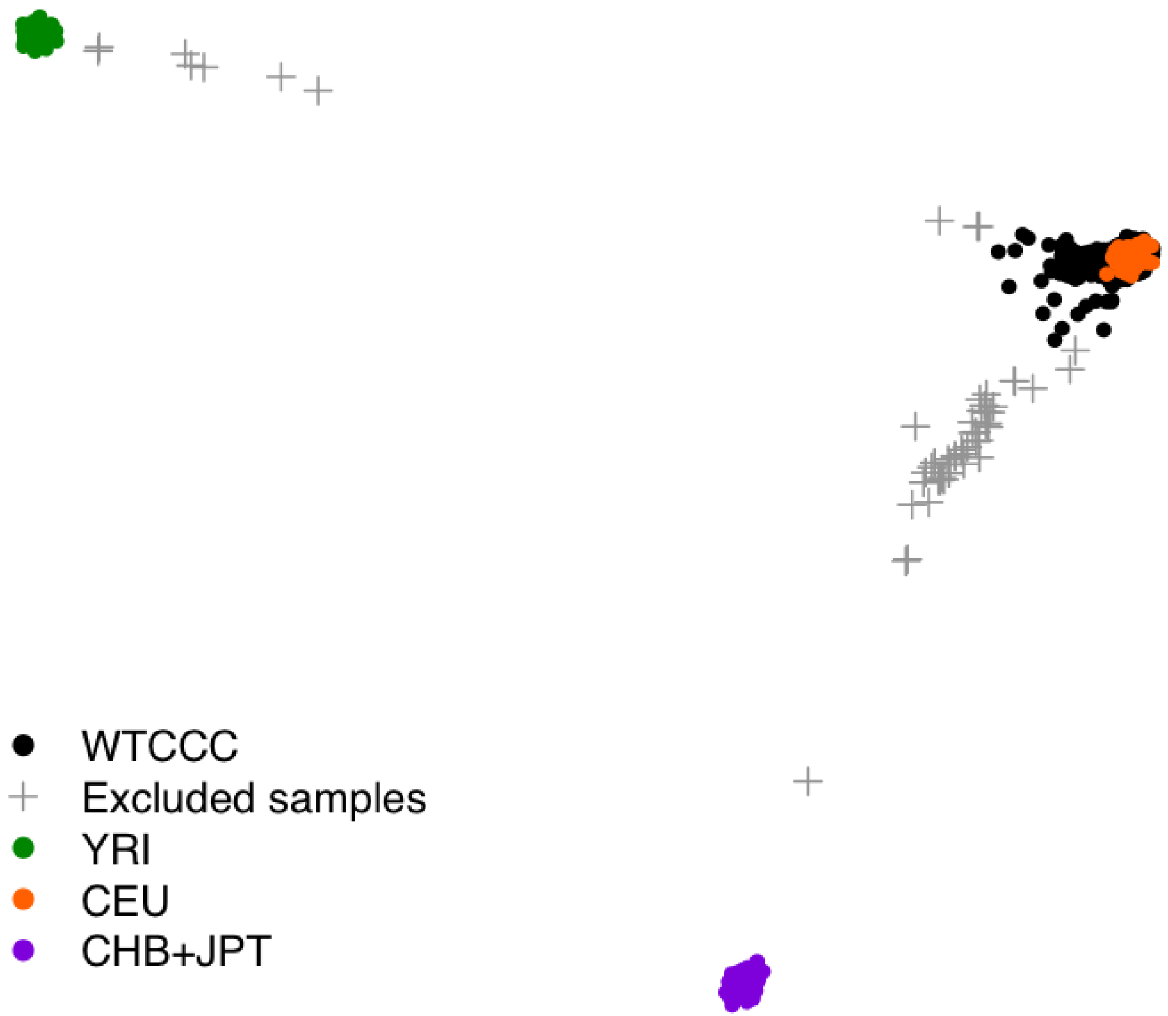
Test locus

Unlinked ‘null’ markers



Stratification → *adjust test statistic*

PCA



Replication

Replication studies should be of sufficient size to demonstrate the effect

Replication studies should conducted in independent datasets

Replication should involve the same phenotype

Replication should be conducted in a similar population

The same SNP should be tested

The replicated signal should be in the same direction

Joint analysis should lead to a lower p value than the original report

Well designed negative studies are valuable

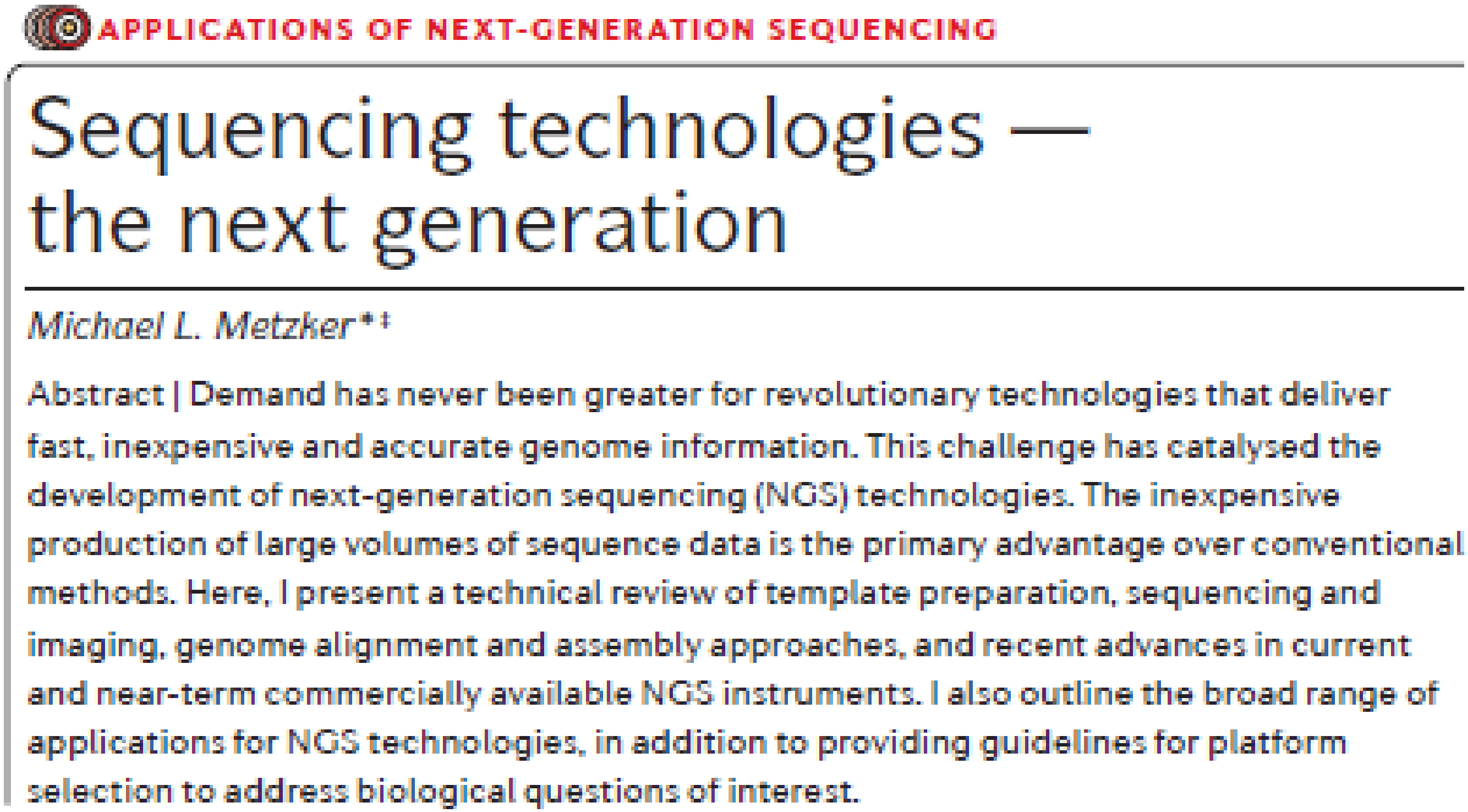
Programs for performing association analysis

- Mx (Neale)
  - Fully flexible, ordinal data
  - Not ideal for large pedigrees or GWAs
- PLINK (Purcell, Neale, Ferreira)
  - GWA
- Haploview (Barrett)
  - Graphical visualization of LD, tagging, basic tests of association
- MERLIN, QTDT (Abecasis)
  - Association and linkage in families

Sequencing and Rare Variants

The case of the missing heritability

When scientists opened up the human genome, they expected to find the genetic components of common traits and diseases. But they were nowhere to be seen. **Brendan Maher** shines a light on six places where the missing loot could be stashed away.



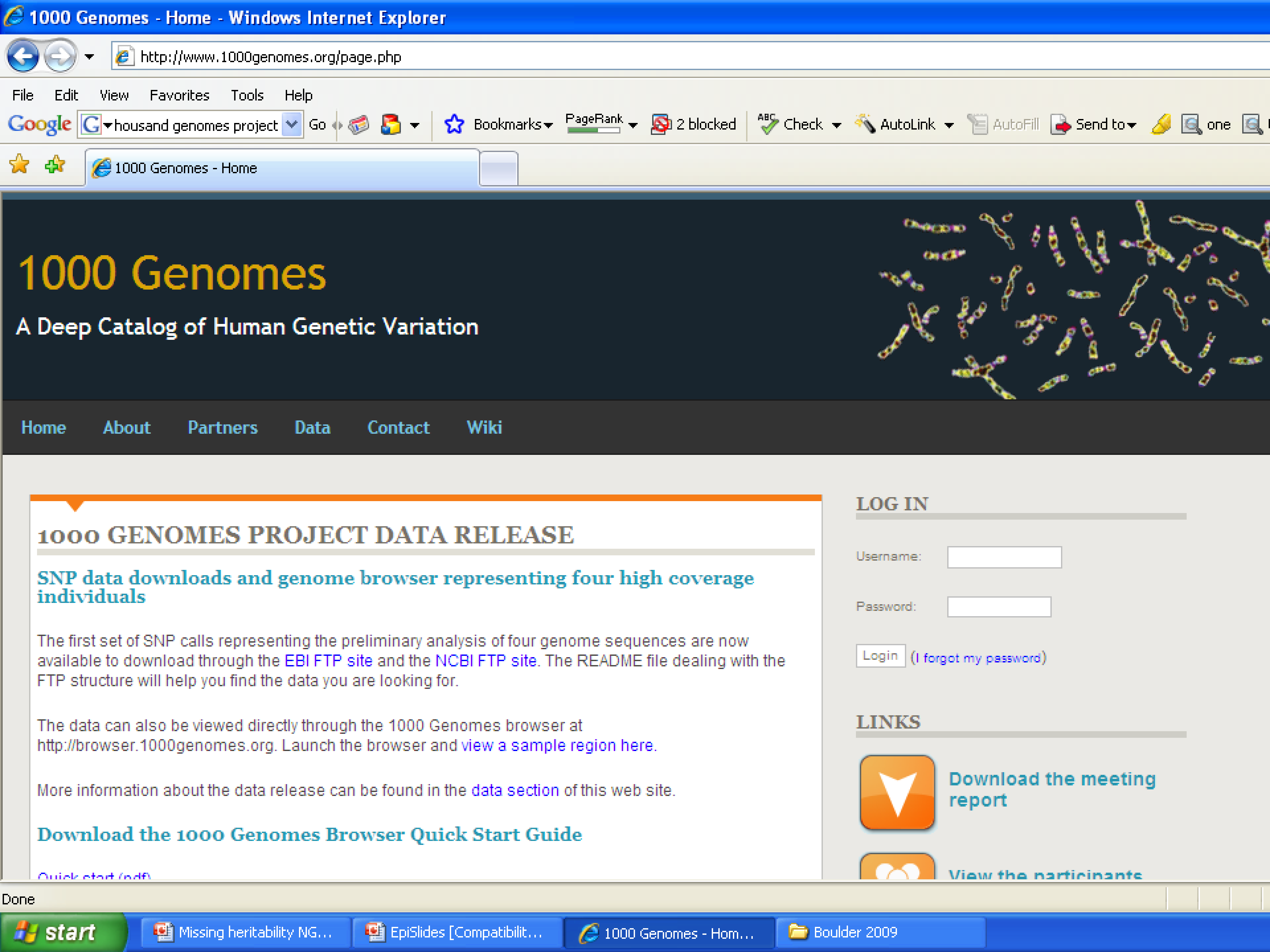
APPLICATIONS OF NEXT-GENERATION SEQUENCING

Sequencing technologies — the next generation

Michael L. Metzker[*,‡]

Abstract | Demand has never been greater for revolutionary technologies that deliver fast, inexpensive and accurate genome information. This challenge has catalysed the development of next-generation sequencing (NGS) technologies. The inexpensive production of large volumes of sequence data is the primary advantage over conventional methods. Here, I present a technical review of template preparation, sequencing and imaging, genome alignment and assembly approaches, and recent advances in current and near-term commercially available NGS instruments. I also outline the broad range of applications for NGS technologies, in addition to providing guidelines for platform selection to address biological questions of interest.

Metzker et al (2010) *Nature Reviews Genetics*



http://www.1000genomes.org/page.php

1000 Genomes

A Deep Catalog of Human Genetic Variation

1000 GENOMES PROJECT DATA RELEASE

SNP data downloads and genome browser representing four high coverage individuals

The first set of SNP calls representing the preliminary analysis of four genome sequences are now available to download through the EBI FTP site and the NCBI FTP site. The README file dealing with the FTP structure will help you find the data you are looking for.

The data can also be viewed directly through the 1000 Genomes browser at http://browser.1000genomes.org. Launch the browser and view a sample region here.

More information about the data release can be found in the data section of this web site.

Download the 1000 Genomes Browser Quick Start Guide

Quick start (pdf)

LOG IN

Username:

Password:

Login (I forgot my password)

LINKS

Download the meeting report

View the participants

Analysis of Rare Variants

- How to combine rare variants?
  - "Ordinary" tests of association won't work
  - Collapse across all SNPs?
- Which SNPs to include?
  - Frequency?
  - Function?
- How to define a region?

Summary

1. Genetic association studies can be used to locate common genetic variants that increase risk of disease/affect quantitative phenotypes

2. Genome-wide association spectacularly successful in identifying common variants underlying complex traits and disease

3. The next challenge is to explain the “missing heritability” in the genome. Genome-wide sequencing and the analysis of rare variants will play a major part in this effort