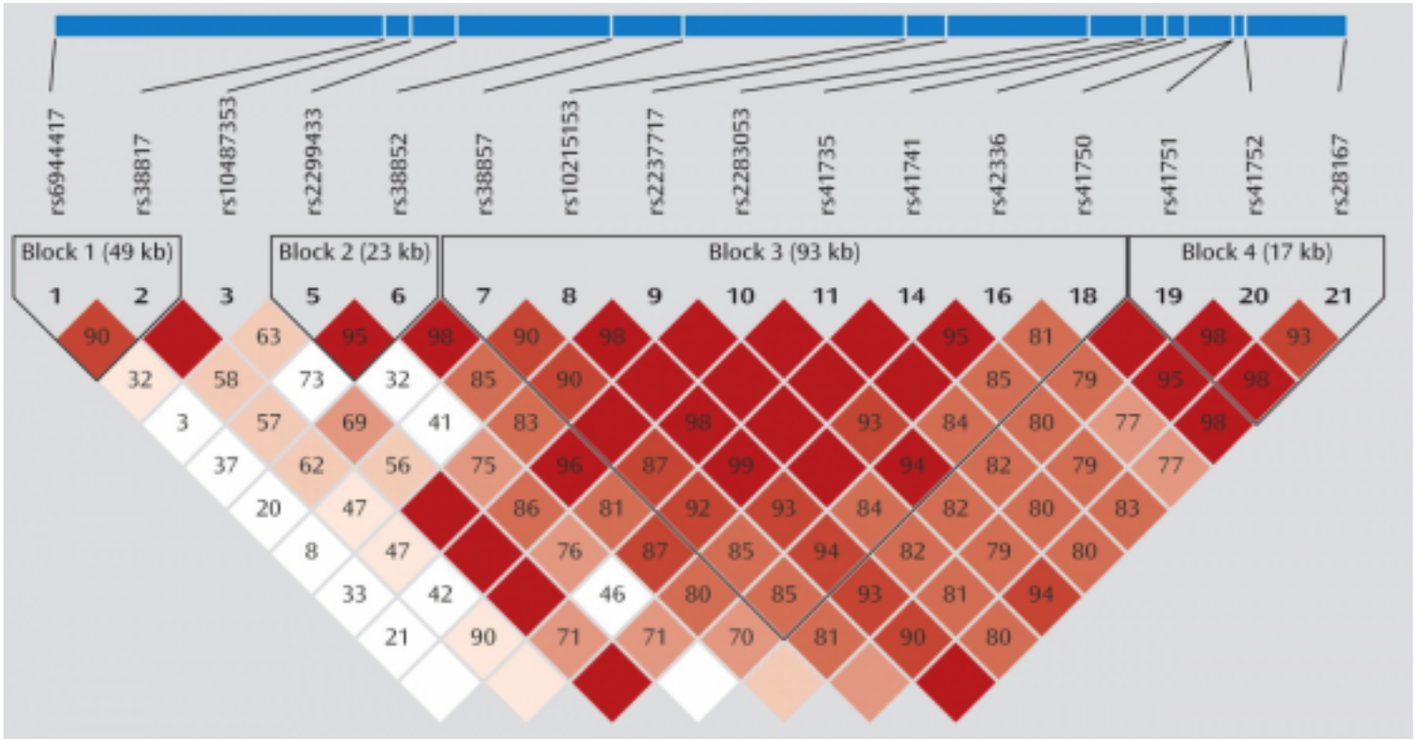
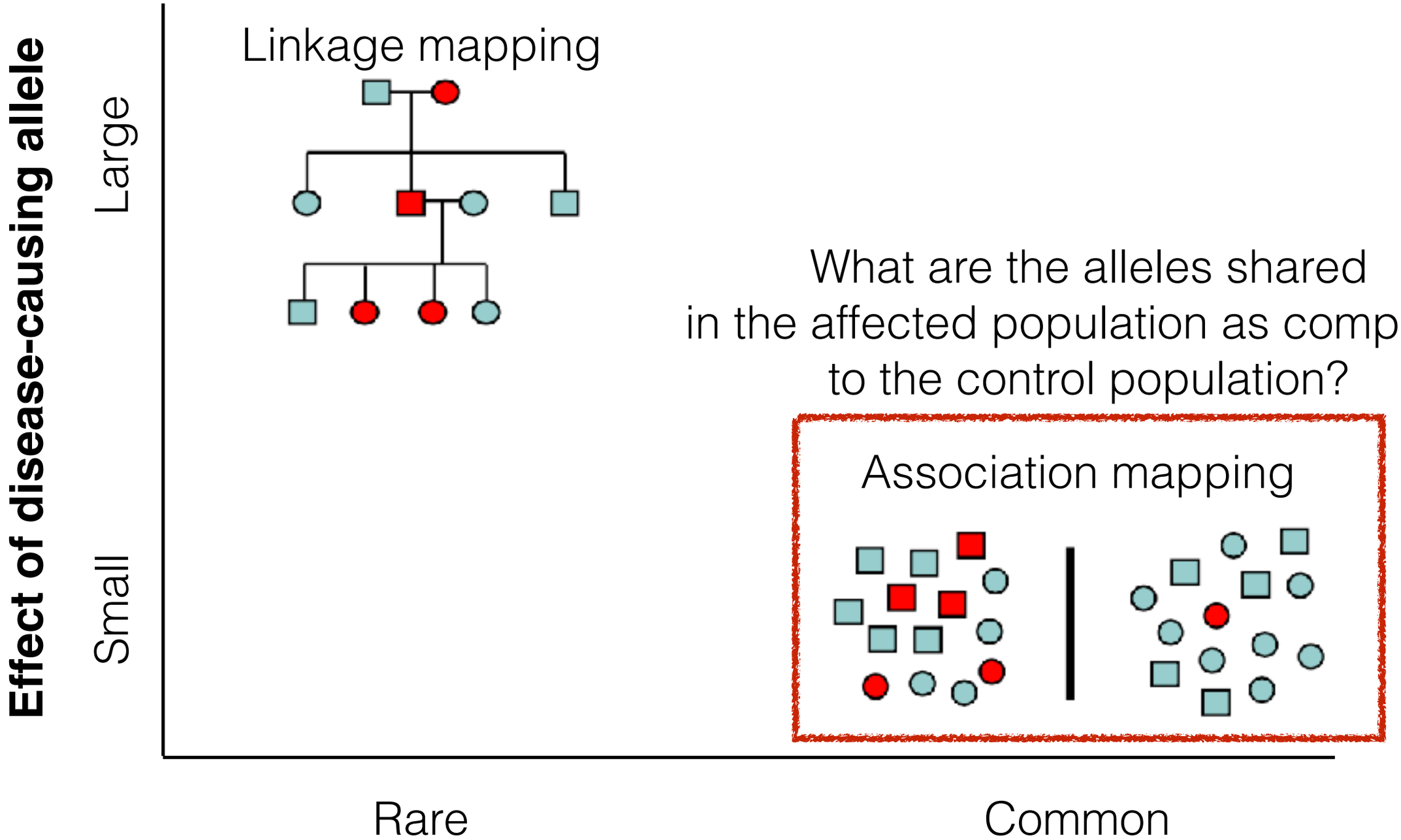
Bio393: Genetic Analysis

Linkage disequilibrium, haplotypes, and GWAS



Human gene mapping has two general flavors

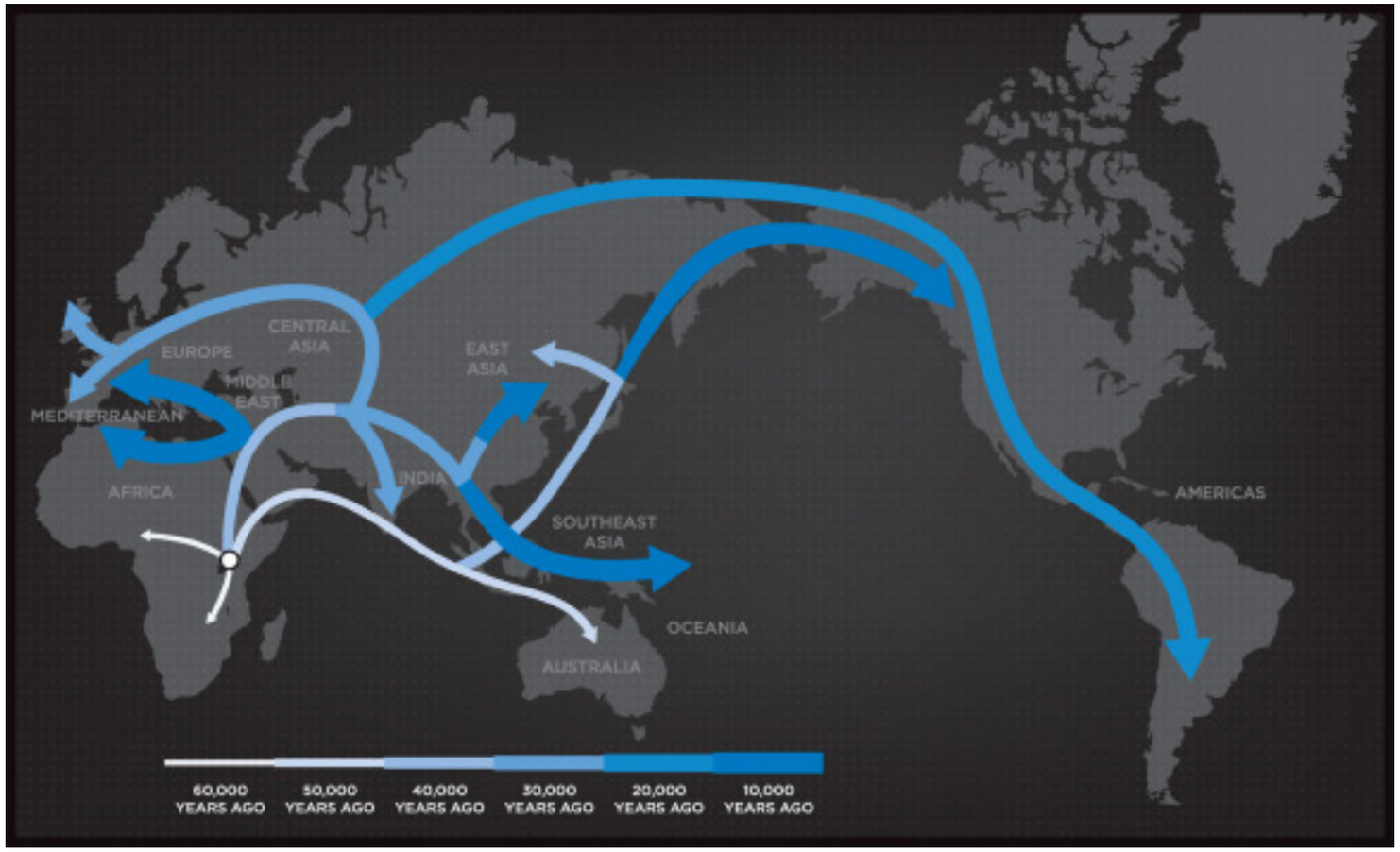


Common variants facilitate genome-wide association (GWA) mapping

The Human Haplotype Map (HapMap) identified
10 million common variants

Do we have to test them all?

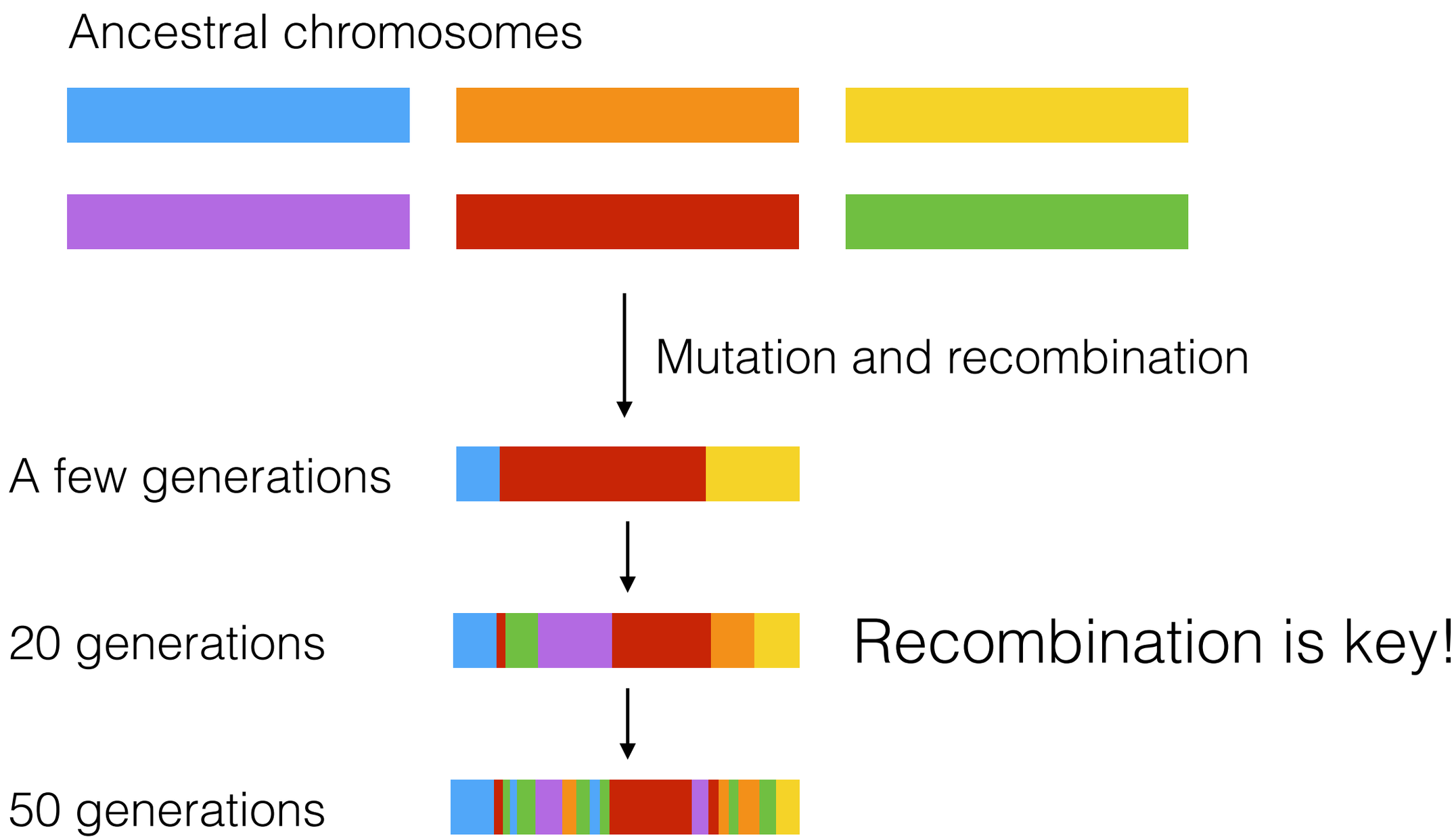
Common variants facilitate genome-wide association (GWA) mapping



Our relatedness means that variants are correlated in populations

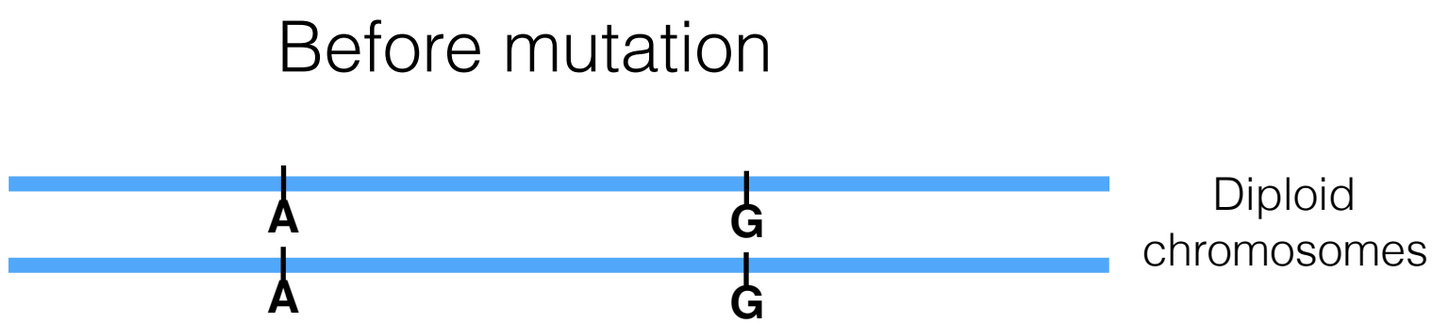
Correlation between variants is called linkage disequilibrium (LD)

Linkage disequilibrium (LD) is the non-random association of alleles at different loci

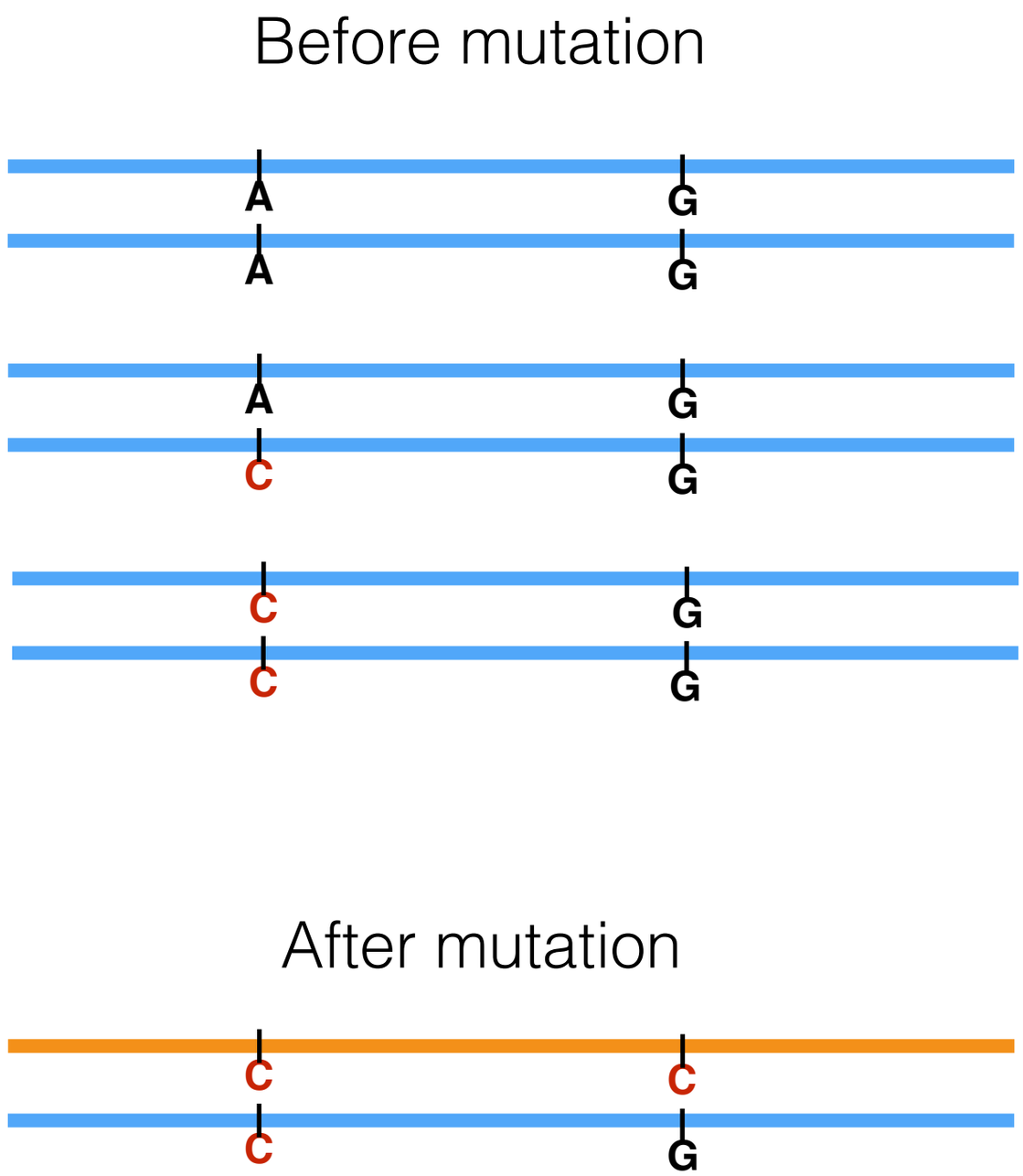


LD makes genotyping easier and cheaper

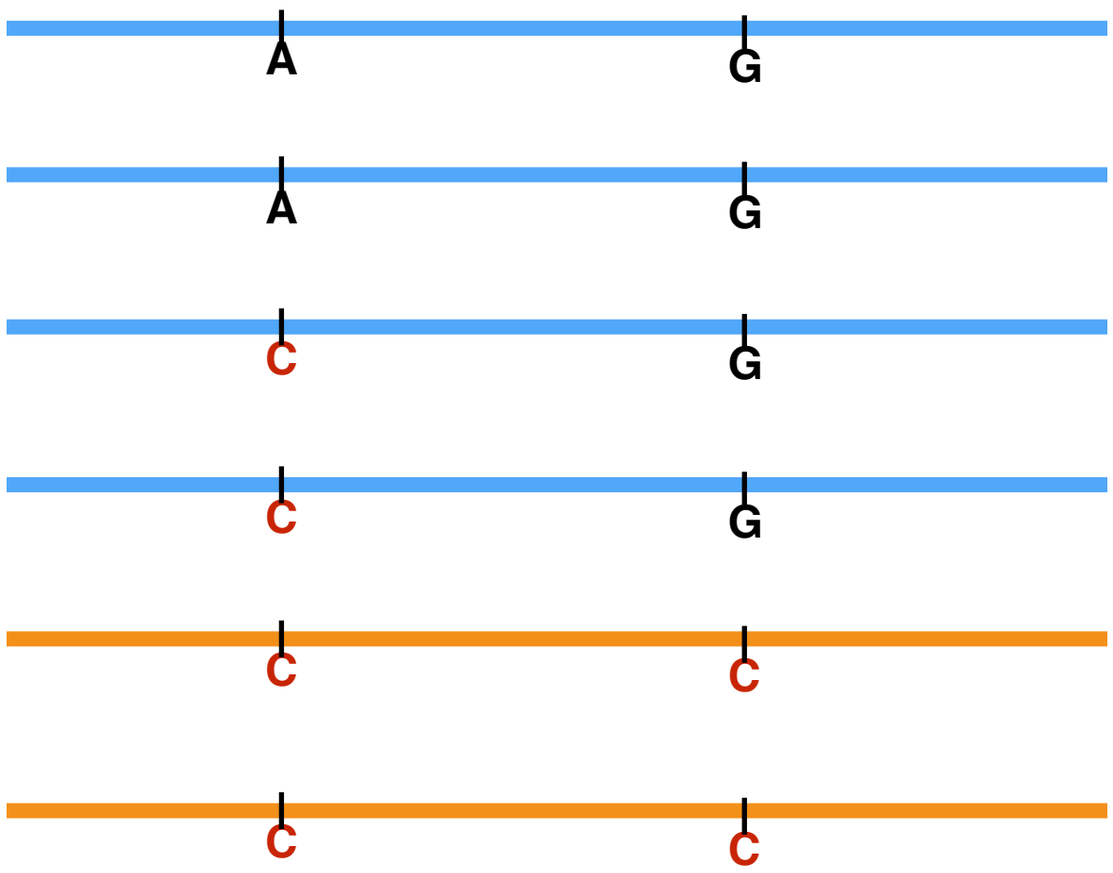
Many alleles that exist today are from ancient mutation events



That allele spreads throughout the population, then another mutation occurs



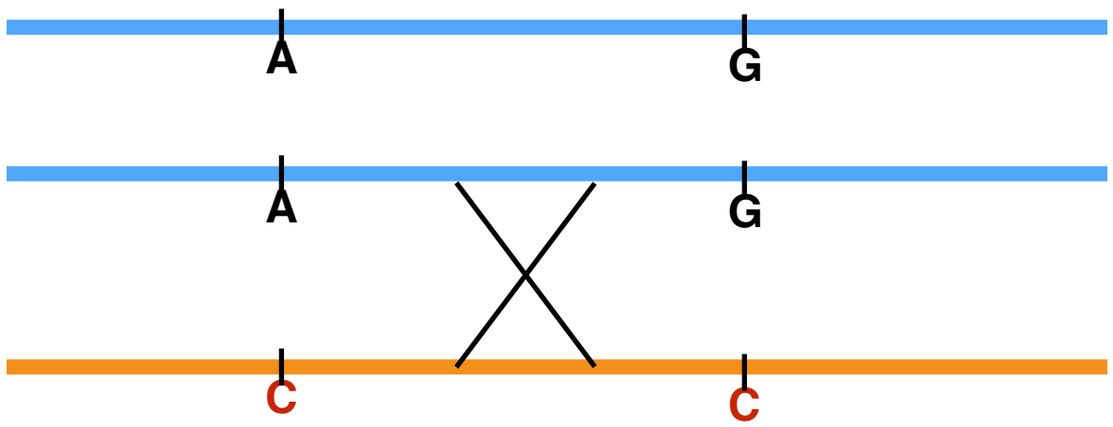
Let’s think about these chromosomes with different arrangements of alleles as haploid gametes



Mutations arose in particular genetic backgrounds,
so not every allelic combination is present

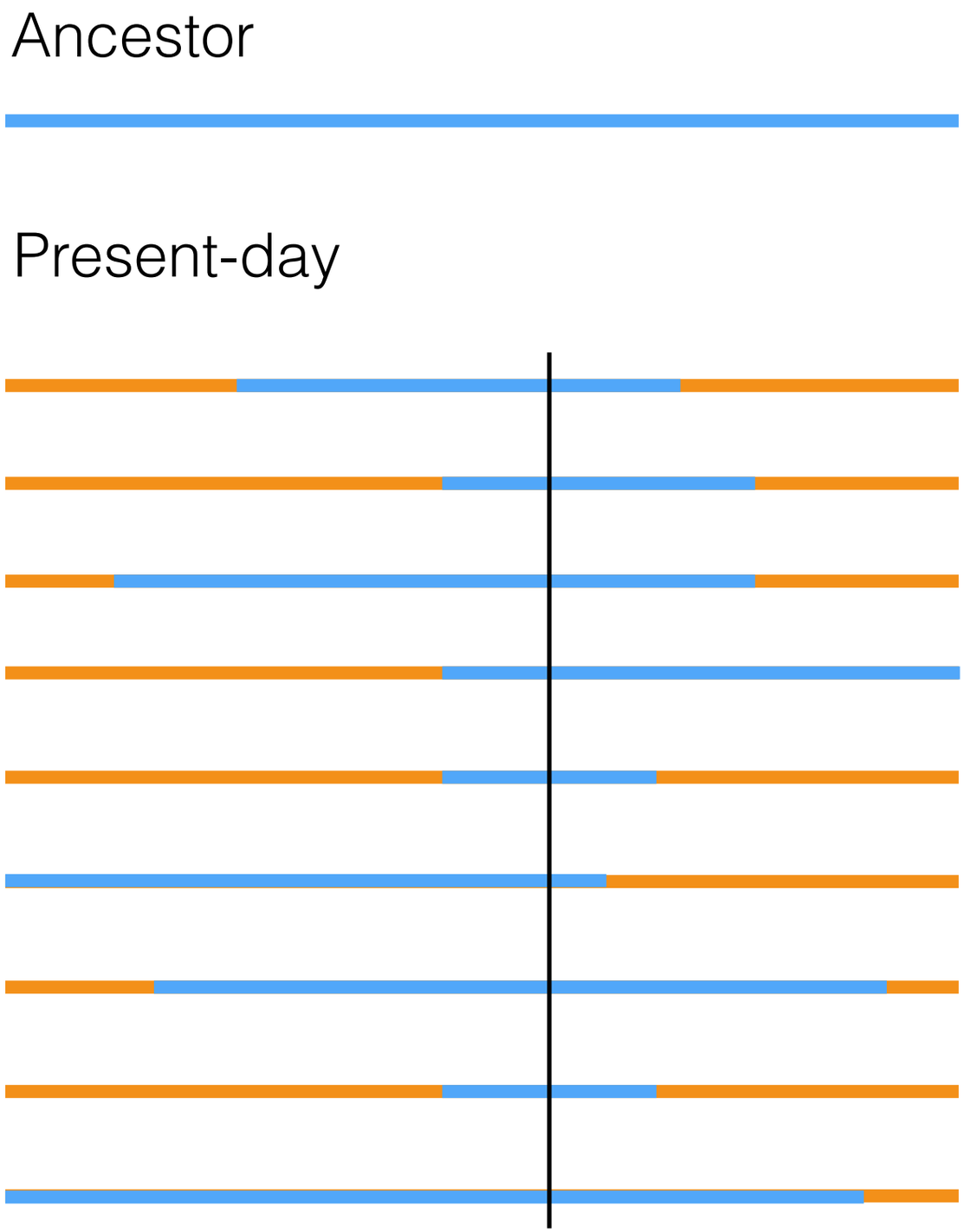
Recombination creates new arrangements of ancestral alleles

Before recombination



After recombination

Linkage disequilibrium is the non-random association of alleles at different loci



Chromosomes are mosaics

Degree of mosaicism depends on:

- Recombination rate
- Mutation rate
- Population size
- Natural selection

Combinations of linked alleles close together reflect ancestral haplotypes

Haplotype frequencies in a population

Let's say we have two linked loci (rs1 and rs2) that each have two alleles (A or a and B or b)

Four combinations exist:

A **B**

A **b**

a **B**

a **b**

p_A = frequency of A in the population or proportion of gametes with A

$p_a = 1 - p_A$

p_B = frequency of B in the population or proportion of gametes with B

$p_b = 1 - p_B$

p_{AB} = frequency of A and B occurring together in the same gamete or frequency of the AB haplotype

These numbers come from genotyping populations

Haplotype frequencies in a population

Let's say we have two linked loci (rs1 and rs2) that each have two alleles (A or a and B or b)

p_A = frequency of A in the population or proportion of gametes with A

$p_a = 1 - p_A$

p_B = frequency of B in the population or proportion of gametes with B

$p_b = 1 - p_B$

p_{AB} = frequency of A and B occurring together in the same gamete
or frequency of the AB haplotype

At equilibrium, the probability of A and B occurring together is the just probability that A and B independently occur in the same gamete

$$p_A * p_B$$

If $p_A * p_B != p_{AB}$, then non-random association or disequilibrium is observed

Haplotype frequencies in a population

p_A = frequency of A in the population or proportion of gametes with A

$p_a = 1 - p_A$

p_B = frequency of B in the population or proportion of gametes with B

$p_b = 1 - p_B$

p_{AB} = frequency of A and B occurring together in the same gamete
or frequency of the AB haplotype

$p_{AB} = p_A * p_B$

Locus rs1	Locus rs2: B	Locus rs2: b	
A	p_{AB}	p_{Ab}	p_A
a	p_{aB}	p_{ab}	p_a
	p_B	p_b	

How to calculate LD?

The Disequilibrium coefficient $D_{rs1\text{-}rs2}$

$D_{rs1\text{-}rs2} = p_{AB} - p_A * p_B$

When in equilibrium, $D_{rs1\text{-}rs2} = 0$

Otherwise, $D_{rs1\text{-}rs2} > \text{or} < 0$

The sign is arbitrary. Set rs1, rs2 to the common alleles.

Range depends on allele frequencies, so comparisons between different pairs of markers are difficult.

How to calculate LD?

The correlation is the preferred term:

$$r^2 = (D_{rs1\text{-}rs2})^2 / (p_A * (1 - p_A) * p_B * (1 - p_B))$$

Remember $D_{rs1\text{-}rs2} = p_{AB} - p_A * p_B$

Ranges between 0 and 1
with 0 being equilibrium and 1 being perfect linkage

How to calculate LD? An example

$r^2 = (D_{rs1\text{-}rs2})^2 / (p_A * (1 - p_A) * p_B * (1 - p_B))$ Remember $D_{rs1\text{-}rs2} = p_{AB} - p_A * p_B$

What is the disequilibrium between two markers rs1 and rs2 with two forms A or a and B or b?

We genotype 500 people to get:

Haplotype	Number
AB	600
Ab	100
aB	200
ab	100

Convert to numbers of alleles into haplotype frequencies:

Haplotype	Number	Frequency
AB	600	0.6
Ab	100	0.1
aB	200	0.2
ab	100	0.1

How to calculate LD? An example

$r^2 = (D_{rs1\text{-}rs2})^2 / (p_A * (1 - p_A) * p_B * (1 - p_B))$ Remember $D_{rs1\text{-}rs2} = p_{AB} - p_A * p_B$

What is the disequilibrium between two markers rs1 and rs2 with two forms A or a and B or b?

Frequencies of haplotypes:

Haplotype	Number	Frequency
AB	600	0.6
Ab	100	0.1
aB	200	0.2
ab	100	0.1

Convert to frequencies of alleles:

$p_A = p(AB) + p(Ab)$
$p_a = 1 - p_A$
$p_B = p(AB) + p(aB)$
$p_b = 1 - p_B$

Allele	Number	Frequency
A	700	0.7
a	300	0.3
B	800	0.8
b	200	0.2

How to calculate LD? An example

$r^2 = (D_{rs1\text{-}rs2})^2 / (p_A * (1 - p_A) * p_B * (1 - p_B))$ Remember $D_{rs1\text{-}rs2} = p_{AB} - p_A * p_B$

What is the disequilibrium between two markers rs1 and rs2 with two forms A or a and B or b?

Convert to frequencies of alleles:

$p_A = p(AB) + p(Ab)$
$p_a = 1 - p_A$
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$p_b = 1 - p_B$

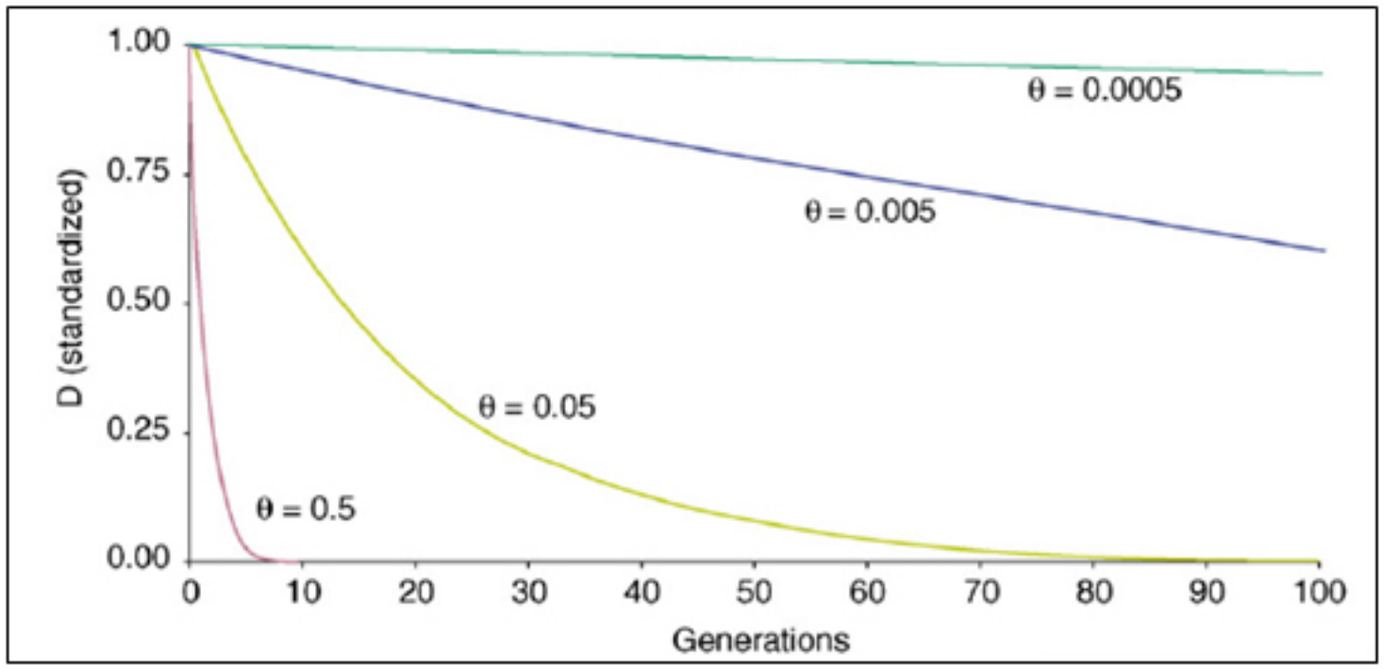
Allele	Number	Frequency
A	700	0.7
a	300	0.3
B	800	0.8
b	200	0.2

Haplotype	Number	Frequency
AB	600	0.6
Ab	100	0.1
aB	200	0.2
ab	100	0.1

$D_{rs1\text{-}rs2} = 0.6 - 0.7 * 0.8 = 0.04$

$r^2 = 0.04^2 / (0.7 * 0.3 * 0.8 * 0.2) = 0.048$

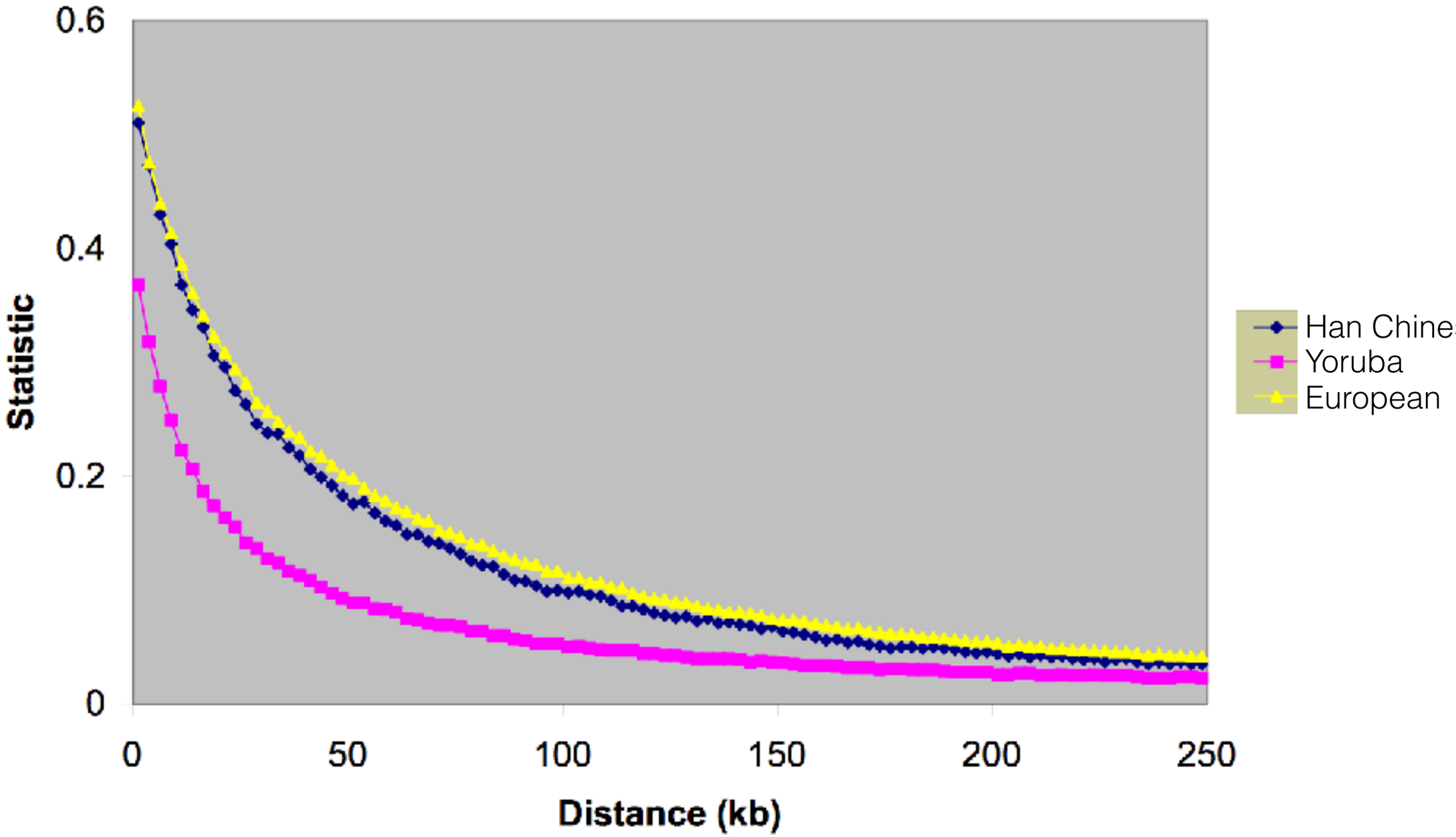
Linkage disequilibrium decreases by distance and generation time



Mackay and Powell 2007

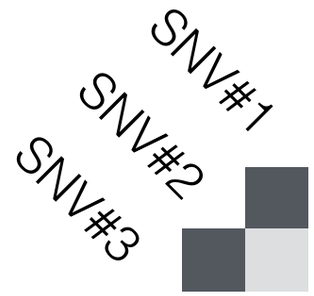
Recombination is key!

Linkage disequilibrium varies among different populations

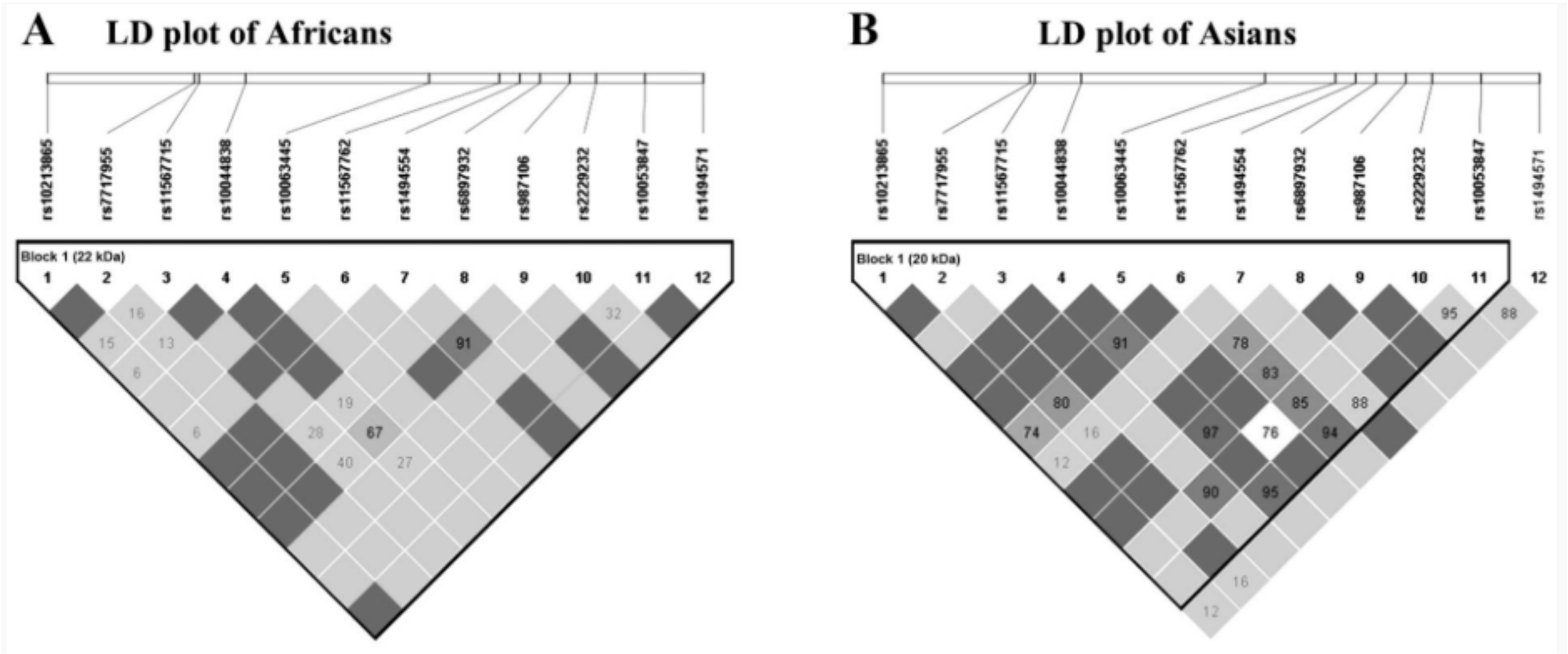


Recombination is key!

Linkage disequilibrium is often shown as a triangle correlation plot



SNV#1 and SNV#2 have high LD
SNV#2 and SNV#3 have high LD
SNV#1 and SNV#3 have low LD

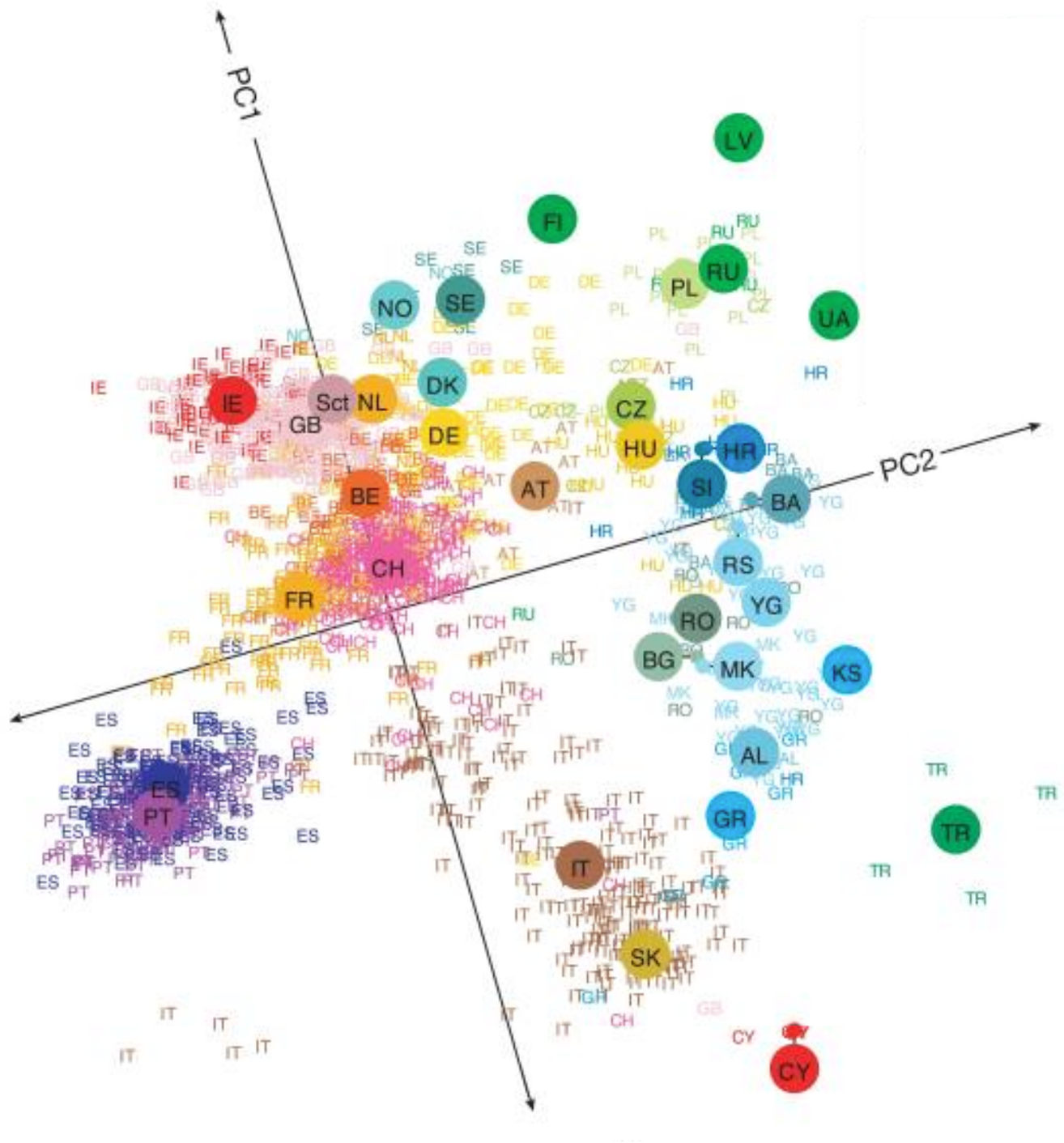


Kim *et al.* Molecular Medicine Reports 2013

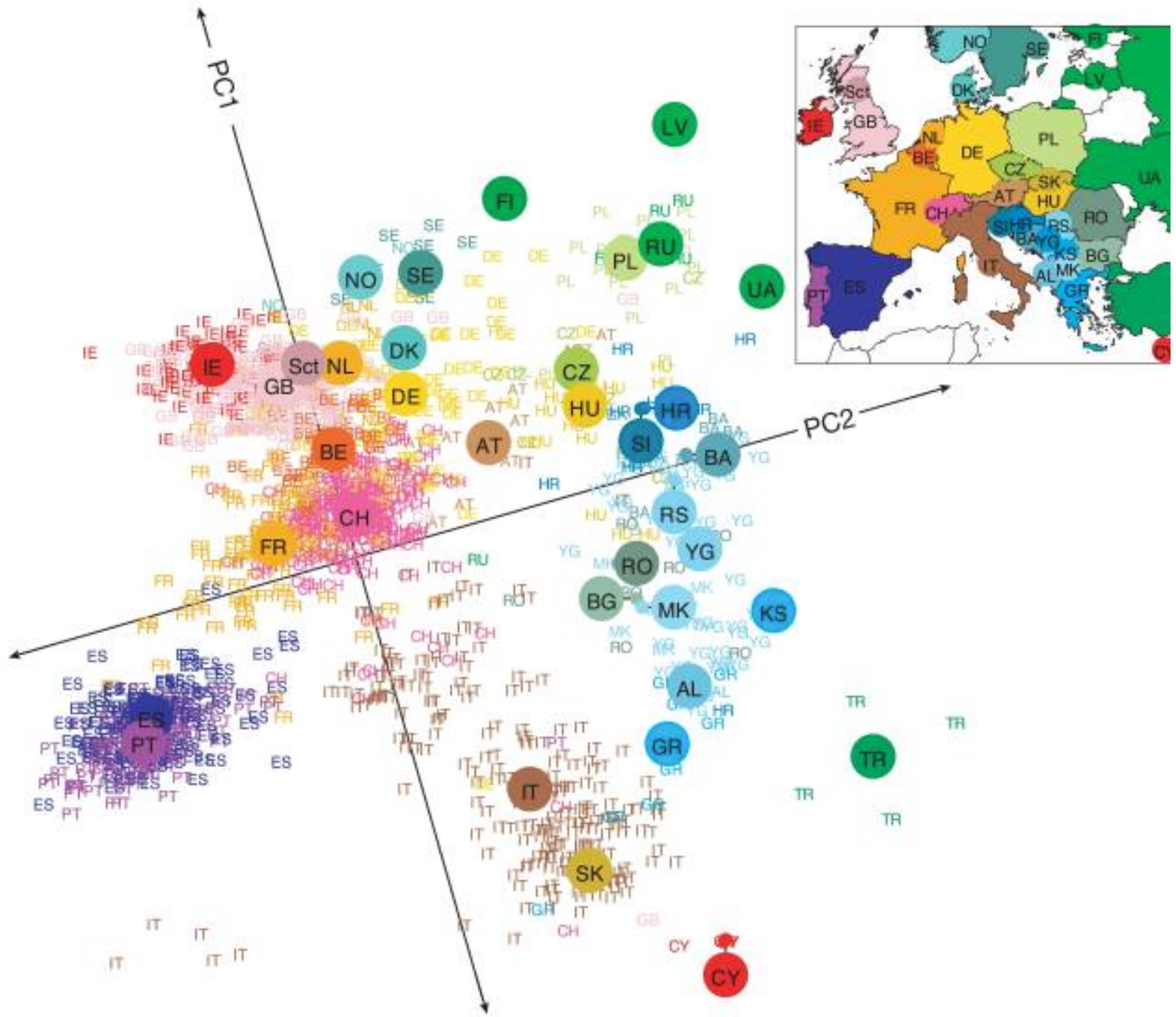
LD leads to population structure - alleles found together in populations

Relatedness of people caused by non-random mating is called population structure (or stratification)

LD leads to population structure - alleles found together in populations



LD leads to population structure - alleles found together in populations



Correlation between marker and disease-causing allele drastically affects how well mappings will work

Big haplotype blocks (long-range LD) = coarse mapping

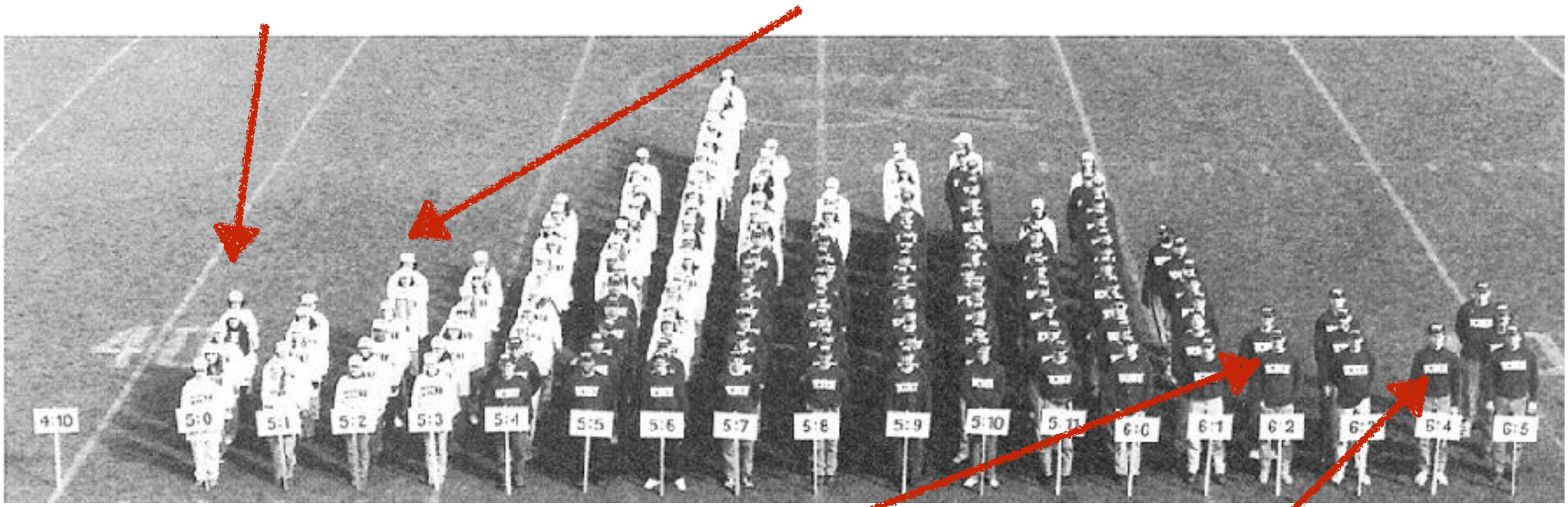
Small haplotype blocks (little LD) = fine mapping

vs.

How many people need to be genotyped?

Genome-wide association studies measure correlation between “tag-SNV” and disease-causing allele

```
CAGCGATAGGCTTTAATGTT  CAGCGATAGGCTTTAATGTT
AGCCCGTTTTATGACCAACG  AGCCCGTTTTATGACCAACG
GGGTTCACAGTGAGCTGTGT  GGGTTCACAGTGAGCTGTGT
```



University of Connecticut, 1997

```
CAGCGATAGGCTTTAATGTT  CAGCGATAGGCTTTAATGTT
AGCCCGTTTGATGACCAACG  AGCCCGTTTGATGACCAACG
GGGTTCACAGTGAGCTGTGT  GGGTTCACAGTGAGCTGTGT
```

Common polymorphims facilitate genome-wide association (GWA) mapping

The Human Haplotype Map (HapMap) identified
10 million common polymorphisms

LD blocks in humans are 20-100 kb

500,000 common variants gives us a SNV every 10 kb

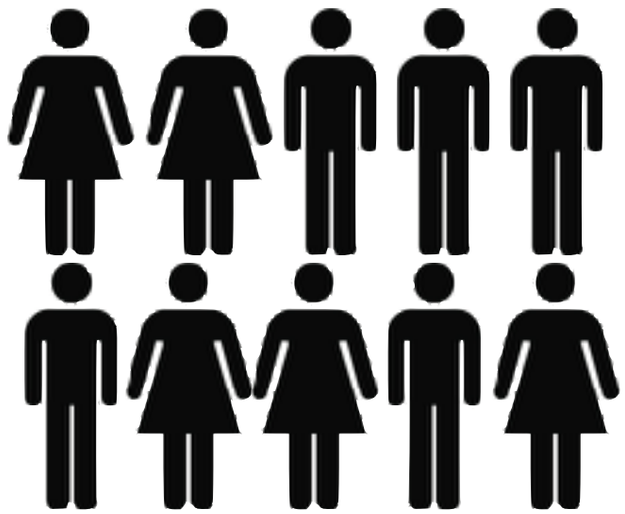
2-10 SNV mark each LD block for the statistical test

Why do 4.3M SNV tests on current arrays?

The set up of a genome-wide association (GWA) mapping

Case-control study design

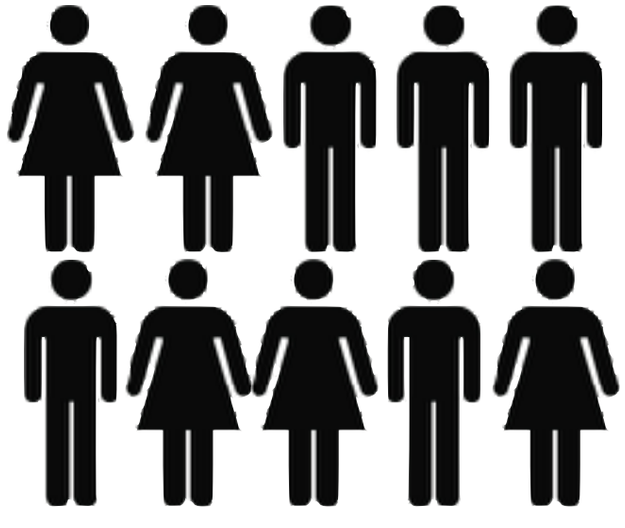
Cases
(People with trait)



Controls
(People without trait)

What alleles do the cases share that the controls lack?

Collect genotype and phenotype data for lots of people

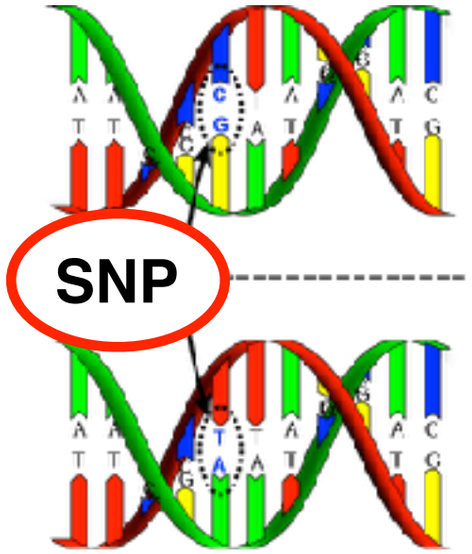


Genotype: SNV arrays (>500k) or sequencing

Phenotype: Measure quantitative values

$250 million spent since 2006 on GWAS

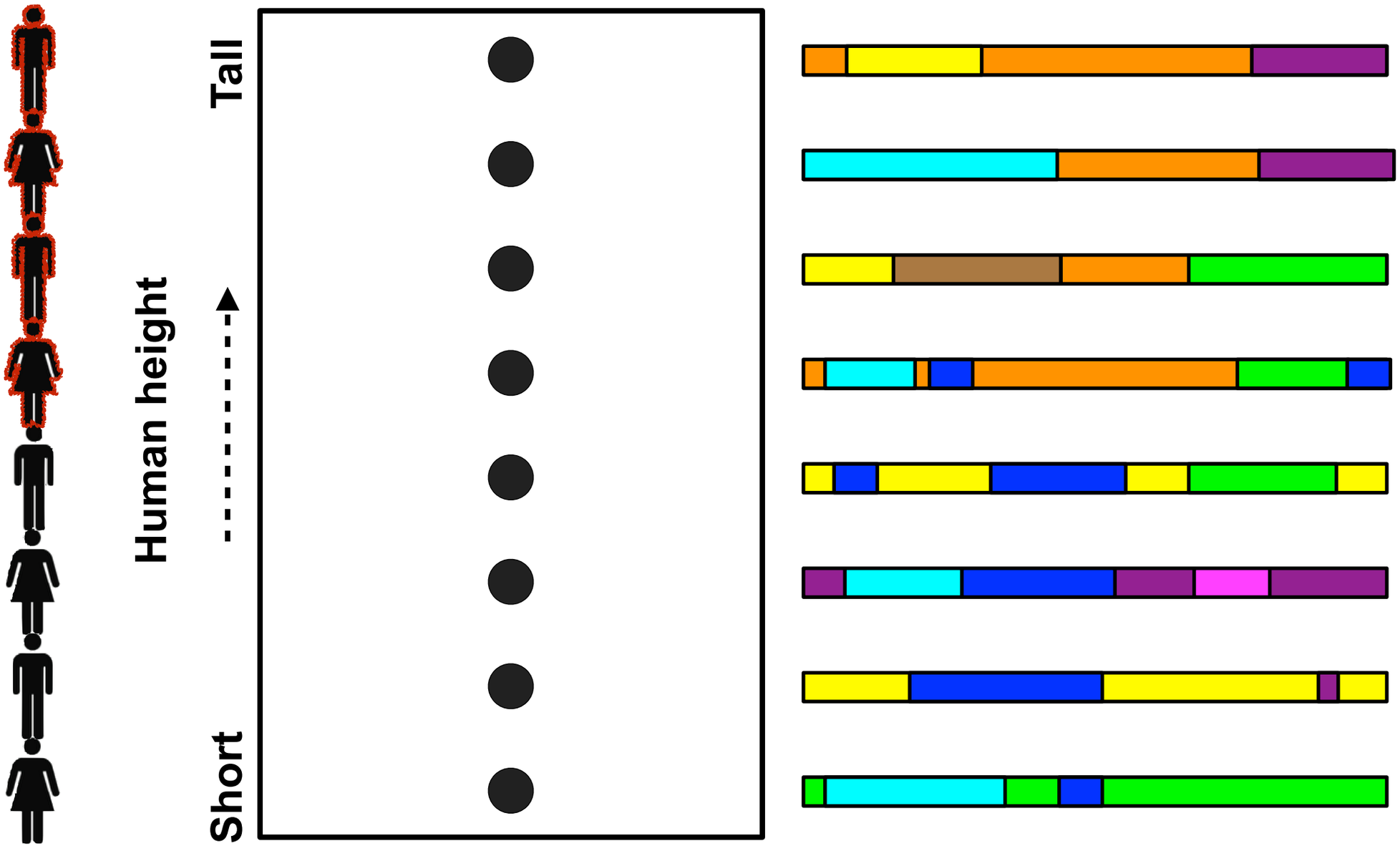
Measure correlation between genetic variation and phenotypic variation in cases and compare to controls



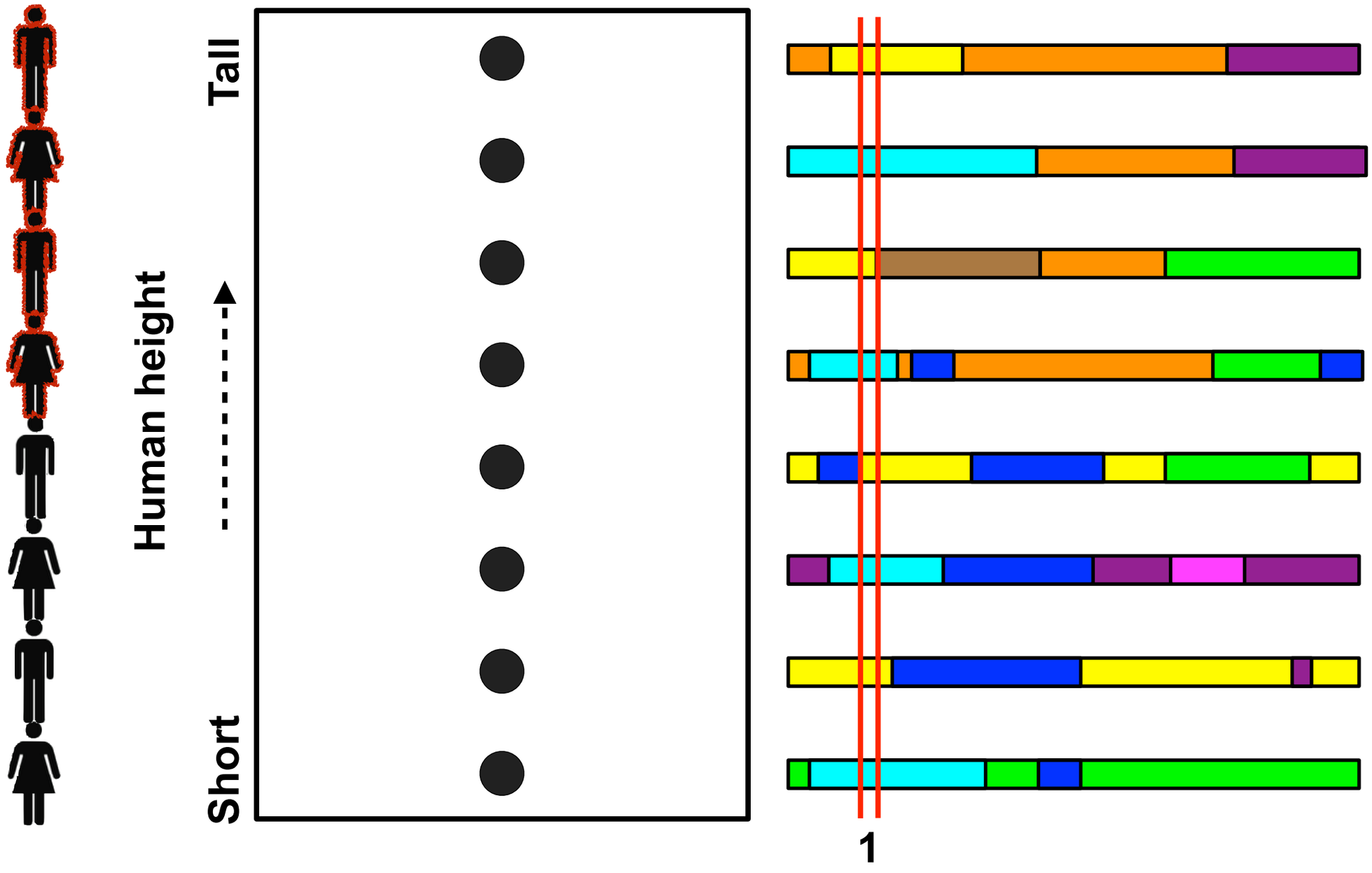
Genetic variation

Phenotypic variation

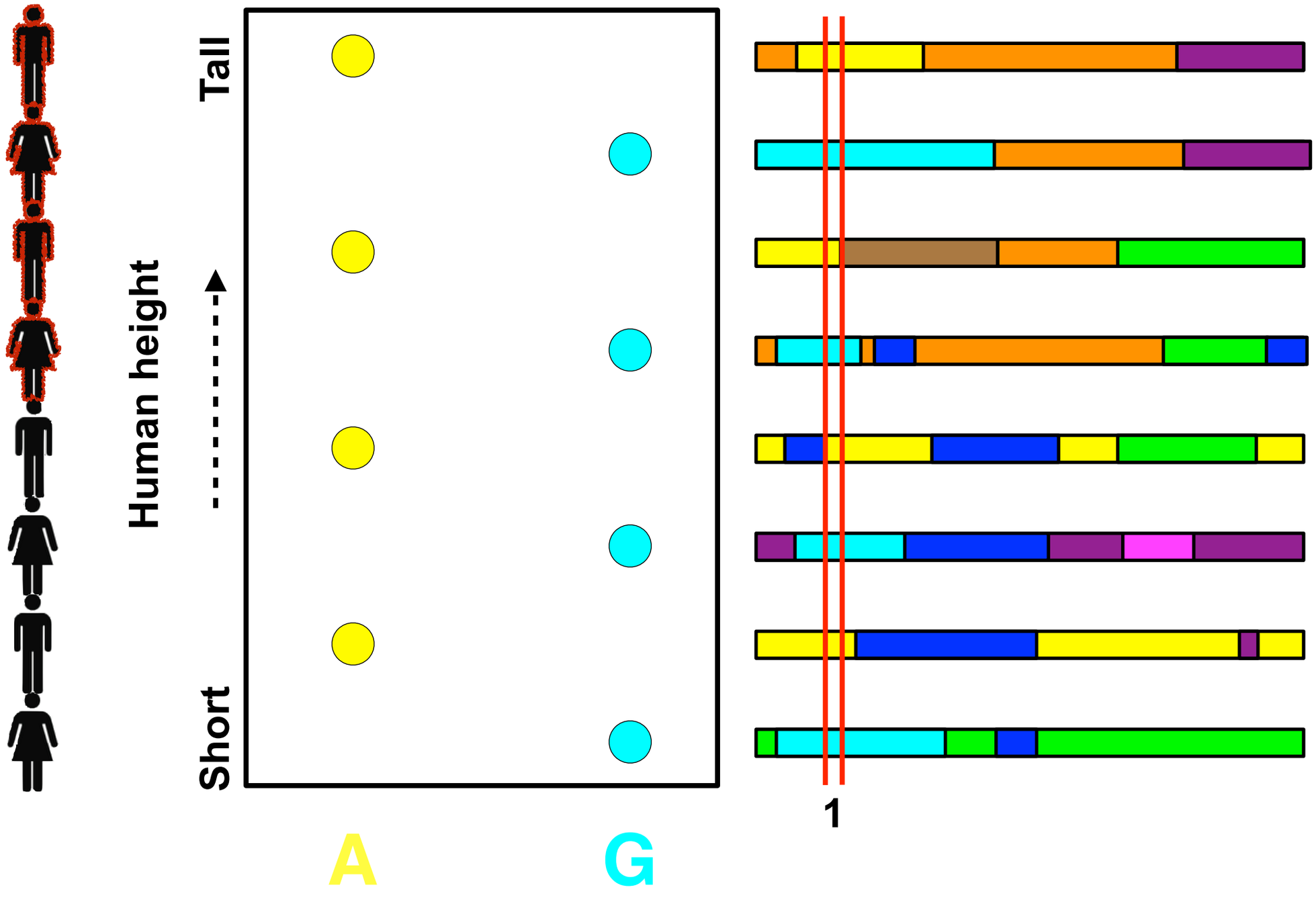
Association mapping:
Correlating genotype with phenotype



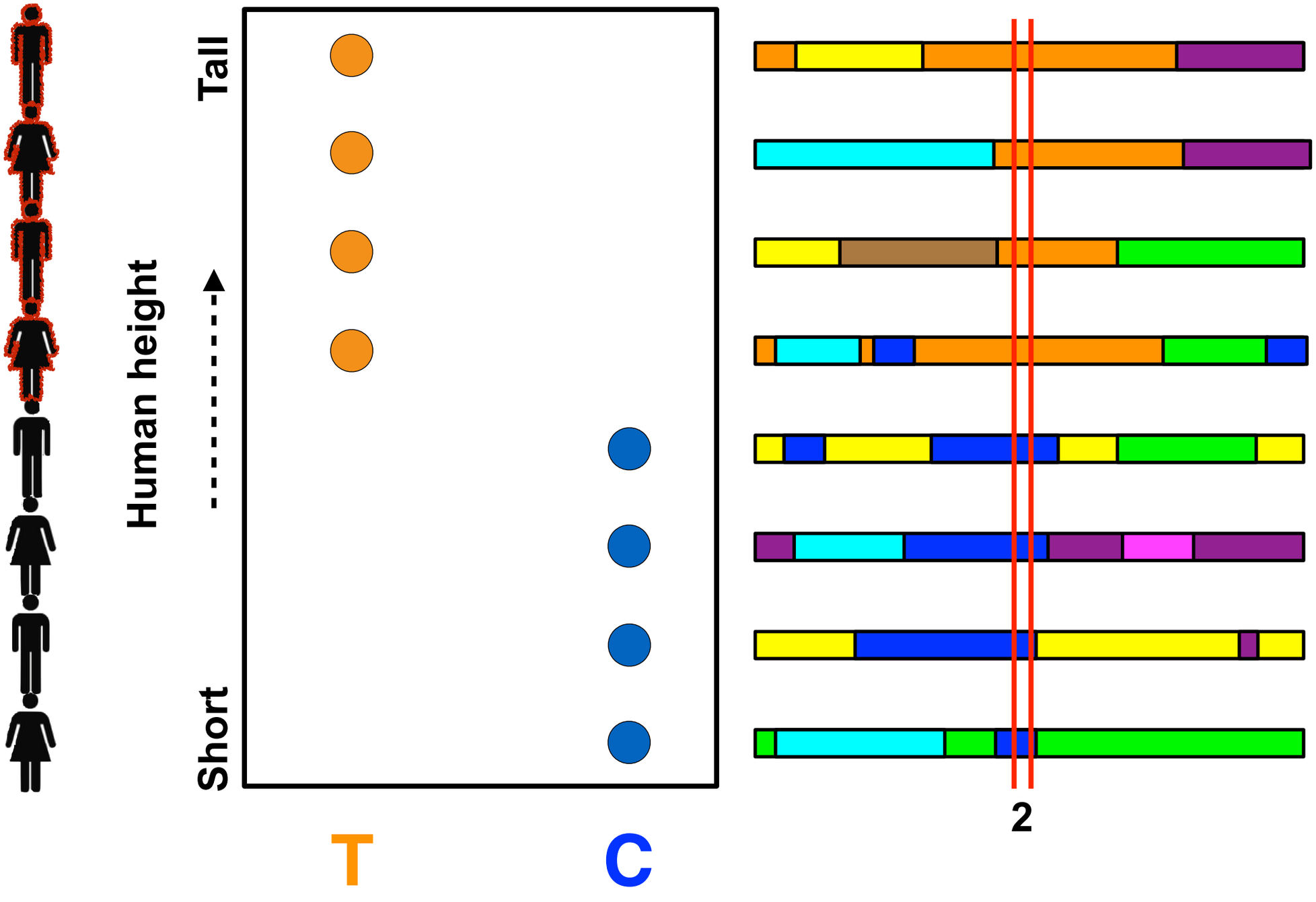
Association mapping:
Correlating genotype with phenotype



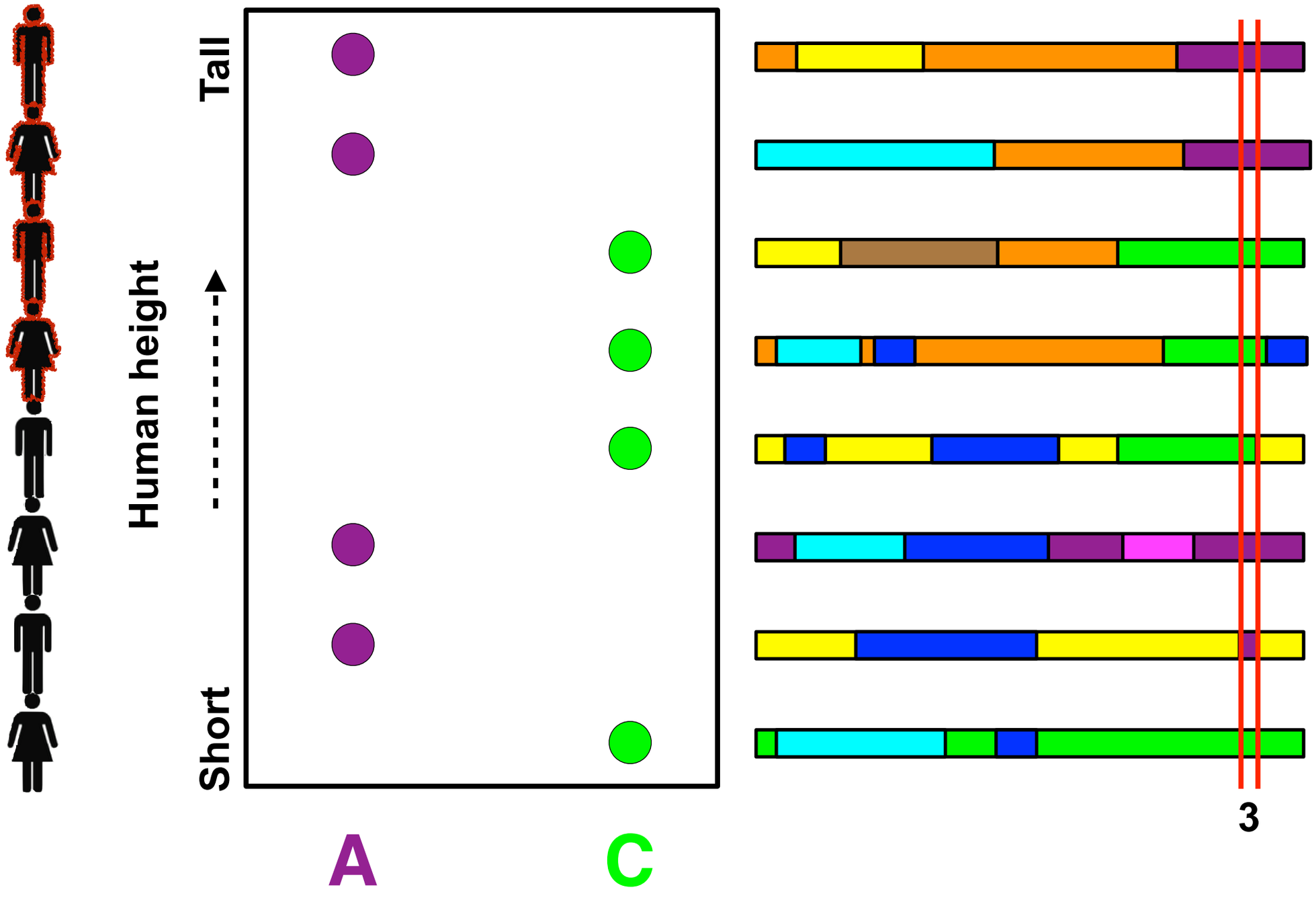
Association mapping:
Correlating genotype with phenotype



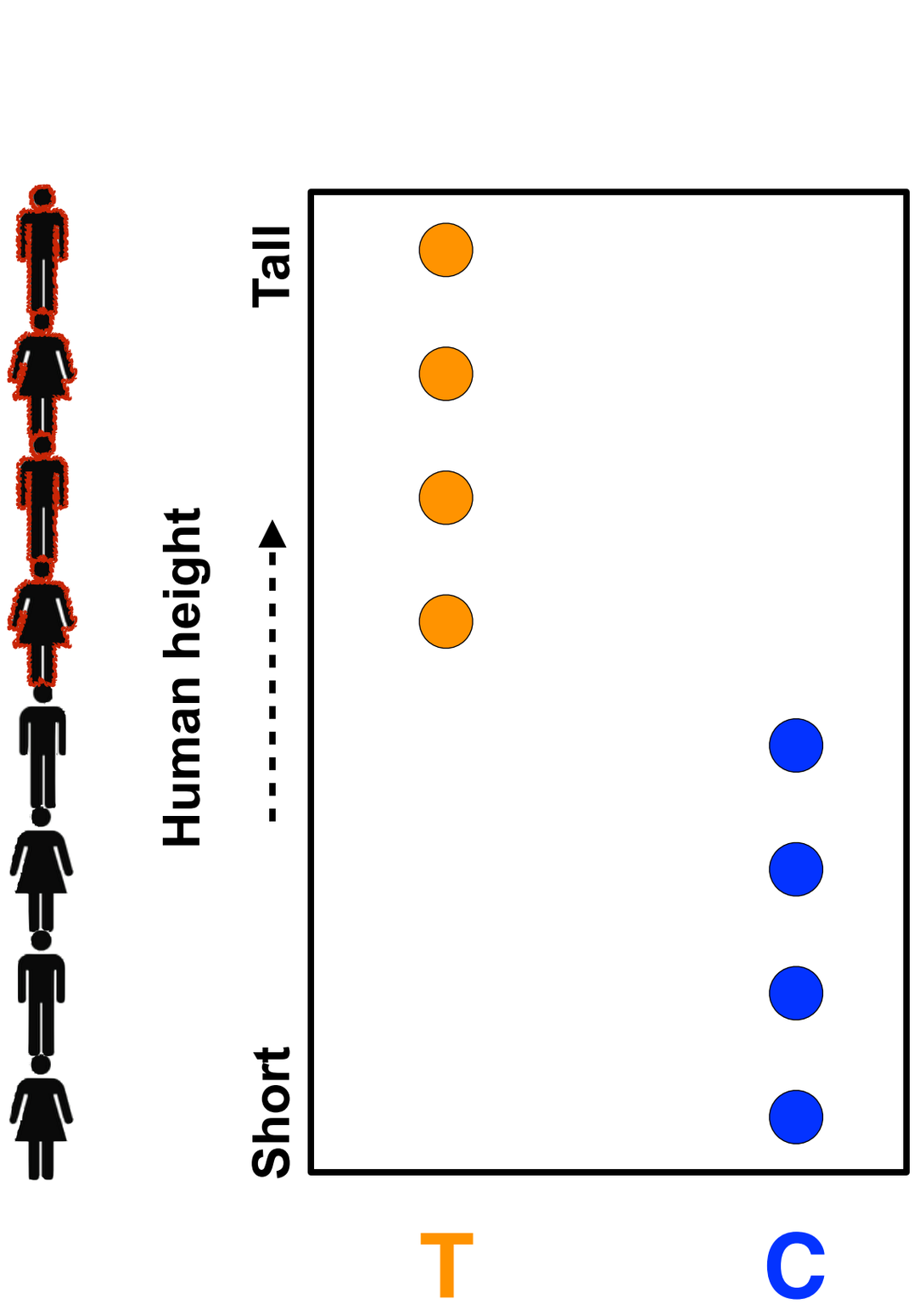
Association mapping:
Correlating genotype with phenotype

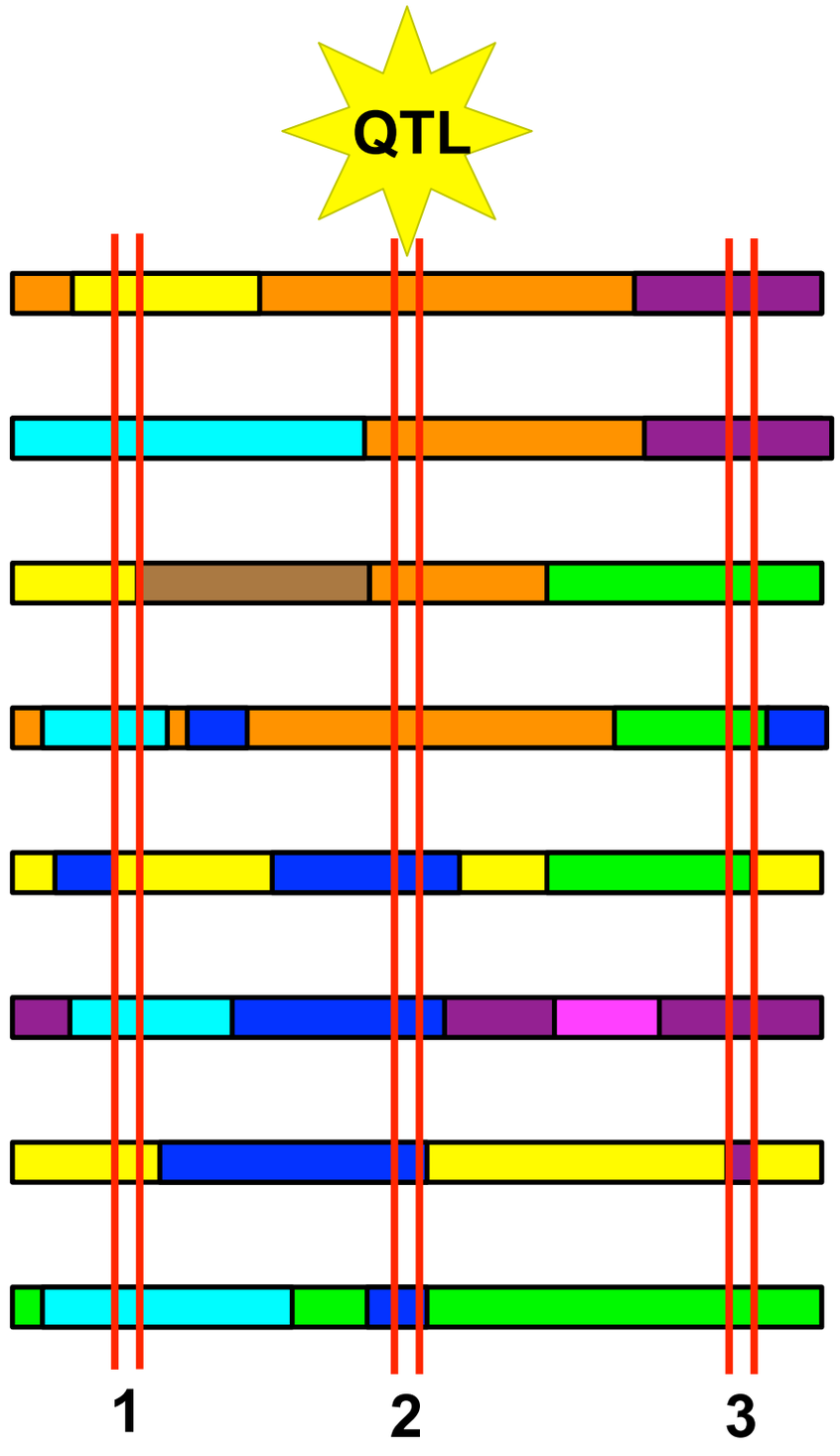


Association mapping:
Correlating genotype with phenotype



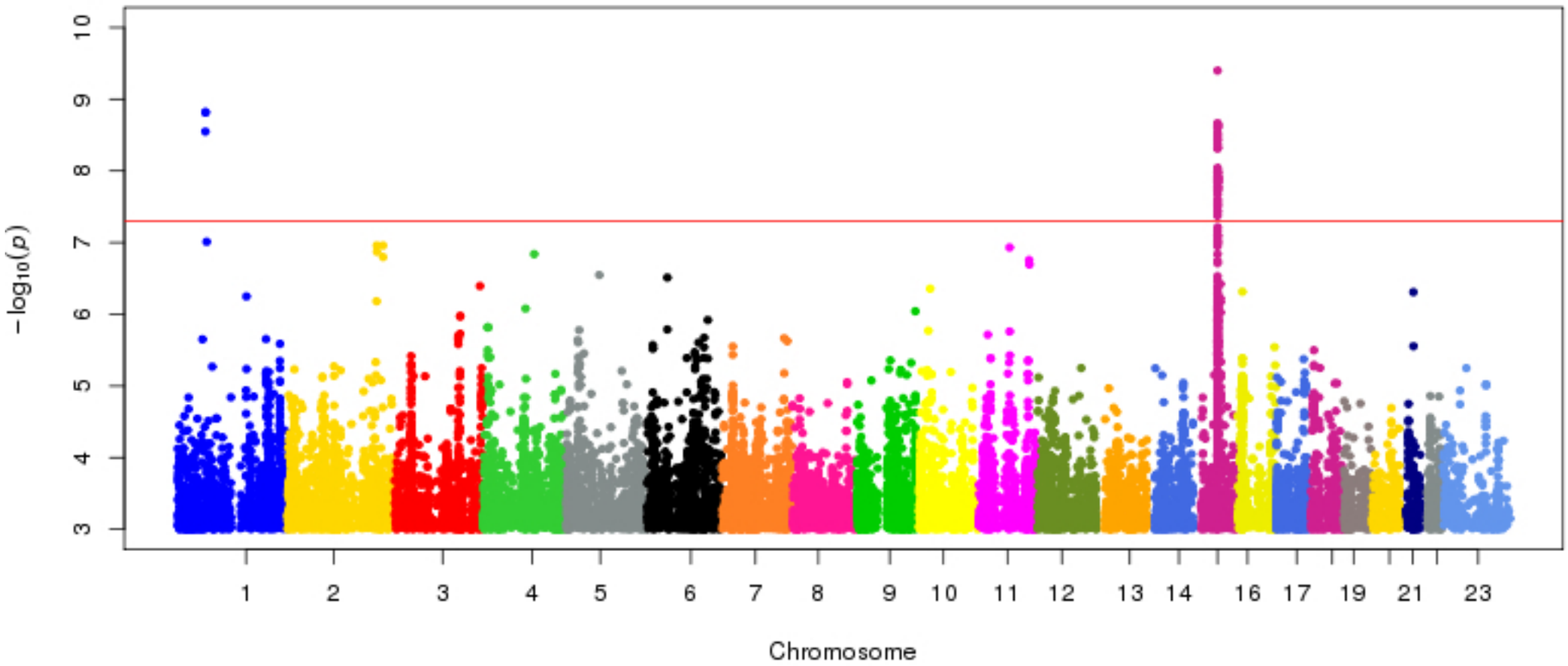
Association mapping:
Correlating genotype with phenotype





QTL=Quantitative Trait Locus

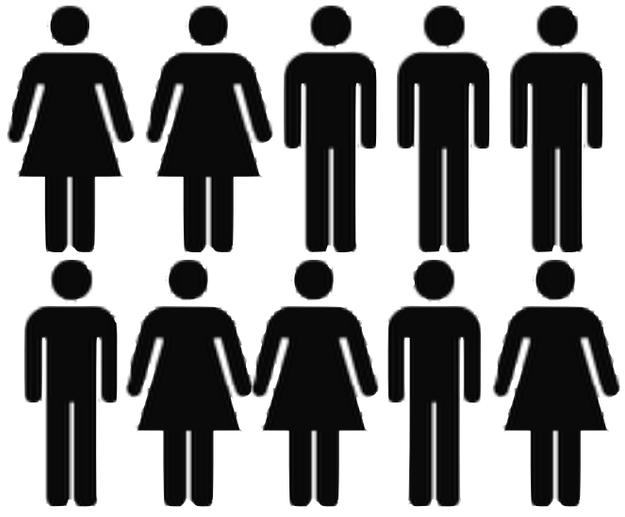
An example Manhattan plot of GWA mapping results



Styrkarsdottir *et al.* Nature 2014

GWAS calculation

4000 Cases



6000 Controls

SNV1
(G or A)

4000 of 8000 (50% G)

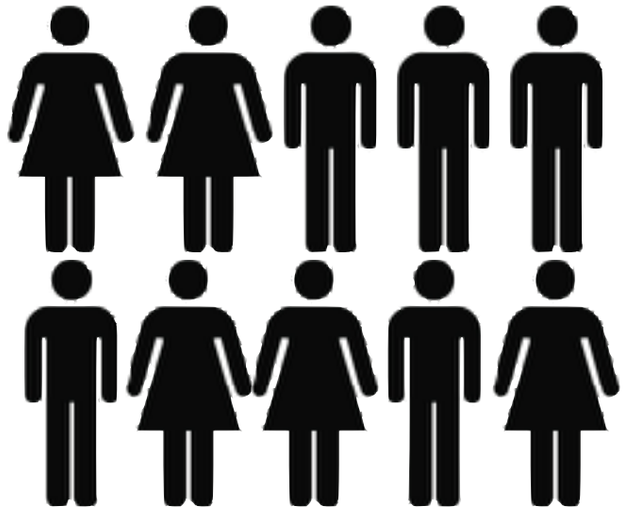
5000 of 12000 (42% G)

	Cases	Controls
G	4000	5000
A	4000	7000

Observed

Expected

GWAS calculation



SNV1
(G or A)

4000 of 8000 (50% G)

5000 of 12000 (42% G)

	Cases	Controls
G	4000	5000
A	4000	7000

Observed

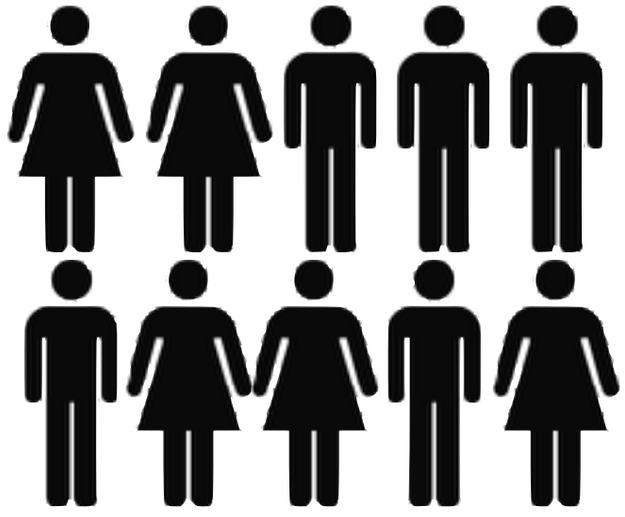
Expected

Pearson's chi-squared test
with one degree of freedom

67.0038 or p-value of 2.71e-16

GWAS calculation

4000 Cases



6000 Controls

SNV1 (G or A)	4000 of 8000 (50% G)	5000 of 12000 (42% G)
SNV2 (T or C)	3200 of 8000 (40% T)	4600 of 12000 (38% T)

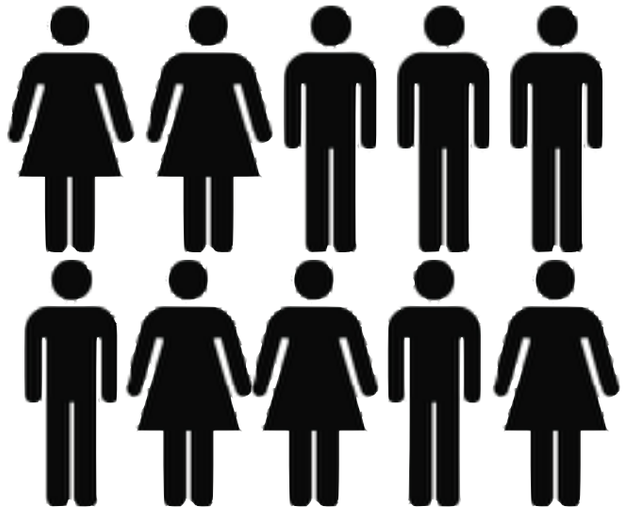
	Cases	Controls
T	3200	4600
C	4800	7400

Observed

Expected

GWAS calculation

4000 Cases



6000 Controls

SNV1 (G or A): 4000 of 8000 (50% G) | 5000 of 12000 (42% G)

SNV2 (T or C): 3200 of 8000 (40% T) | 4600 of 12000 (38% T)

	Cases	Controls
T	3200	4600
C	4800	7400

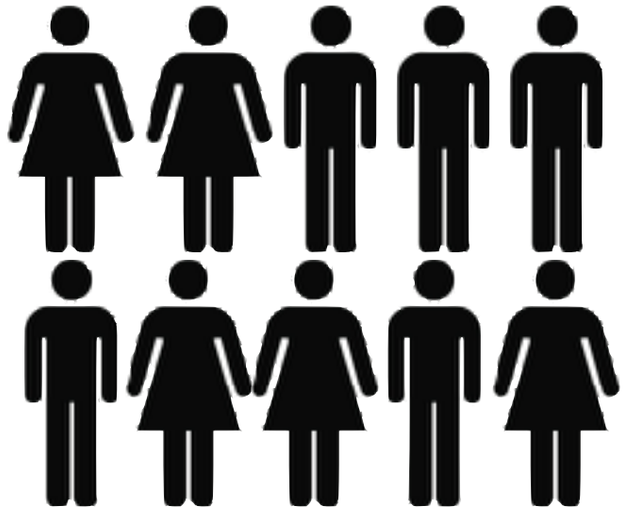
Observed | Expected

Pearson's chi-squared test with one degree of freedom

2.7327 or p-value of 0.09831

GWAS calculation

4000 Cases



6000 Controls

SNV1 (G or A) 4000 of 8000 (50% G) 5000 of 12000 (42% G)

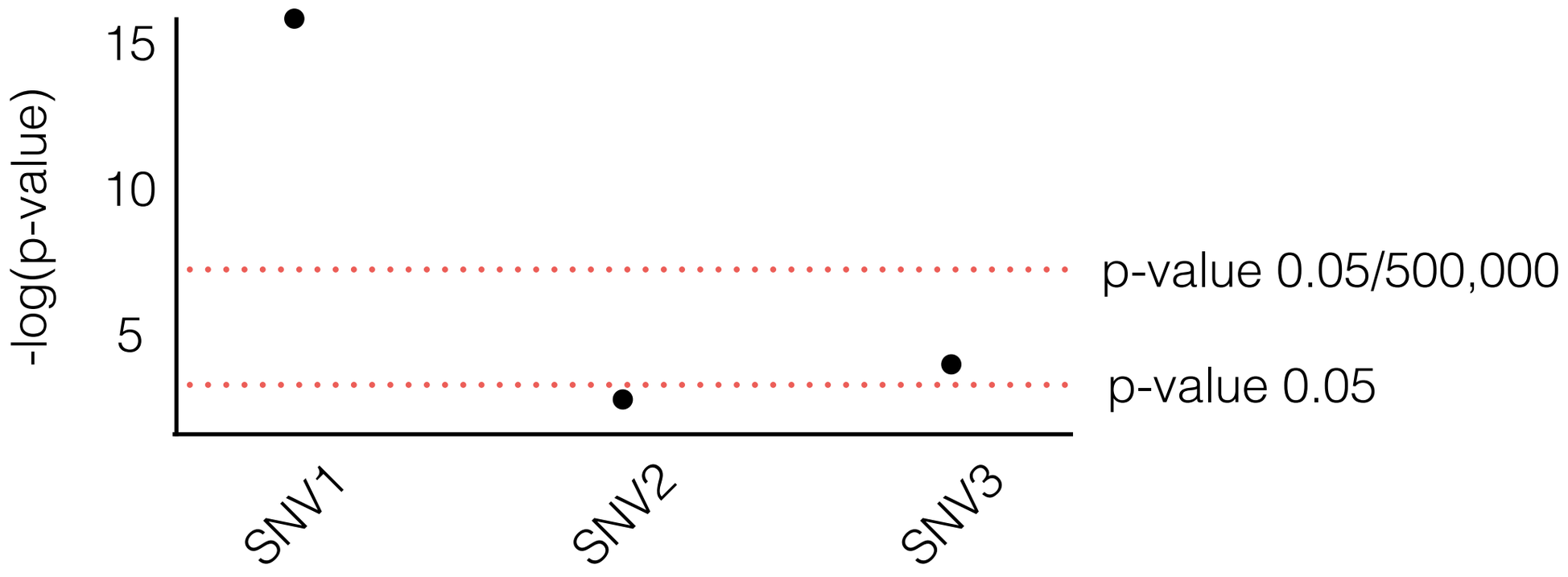
SNV2 (T or C) 3200 of 8000 (40% T) 4600 of 12000 (38% T)

SNV3 (C or A) 3600 of 8000 (45% C) 5000 of 12000 (40% C)

	Cases	Controls
C	3600	5000
A	4400	7000

10.7443 or p-value of 0.001046

GWAS results



500,000 SNVs across the whole genome

Carlo Bonferroni

500,000 tests with a p-value of 0.05 means
that we would reject the null hypothesis
for 25,000 SNVs by chance

Bonferroni correction 0.05 / 500,000 or 1e-7

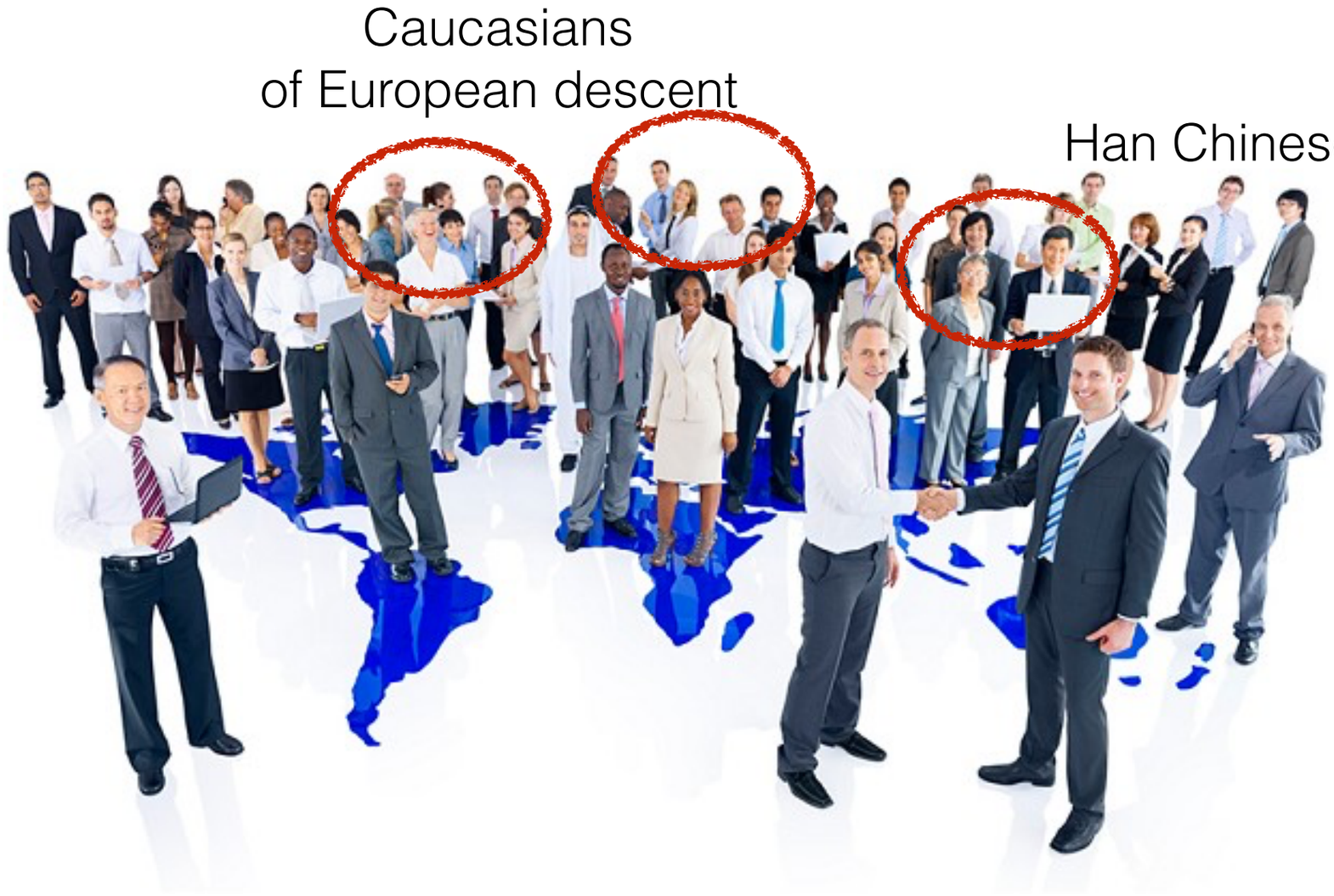
Three possibilities for the results of any GWA mapping

1. Marker is the *functional variant*

2. Marker is in *linkage disequilibrium* with functional variant

3. Marker is associated because of *population relatedness (population structure)*

GWA mapping within groups and replication



GWA mapping works best within a related population

The mapping *might* be replicated in different populations