

# BIOL 350: Bioinformatics

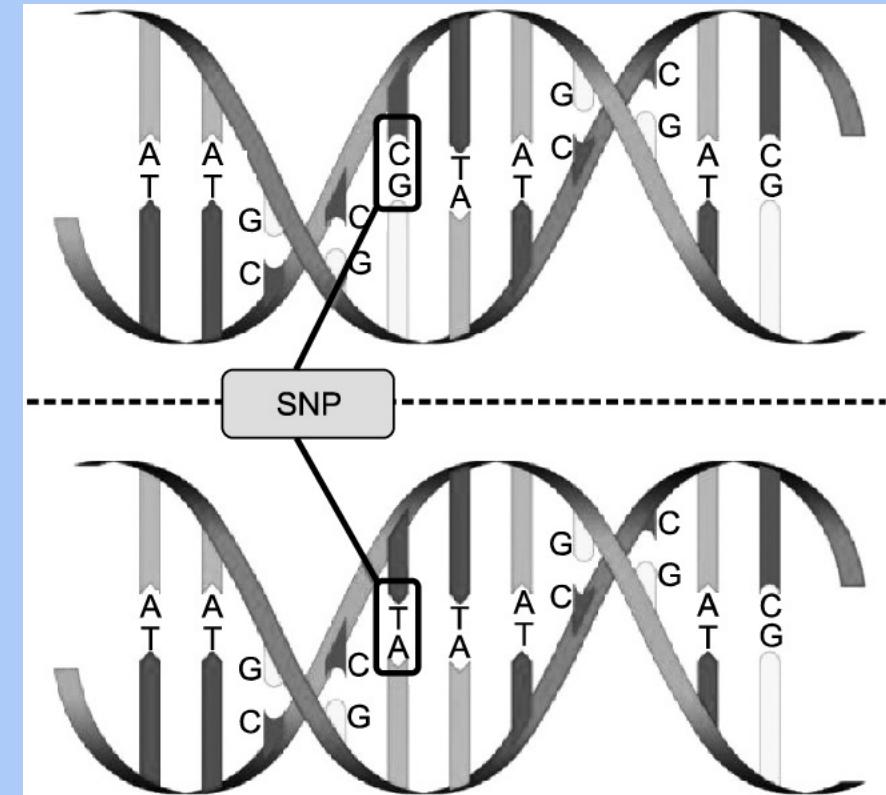
## Introduction to genetic association studies

# What is a SNP?

Polymorphisms and their role in genetics

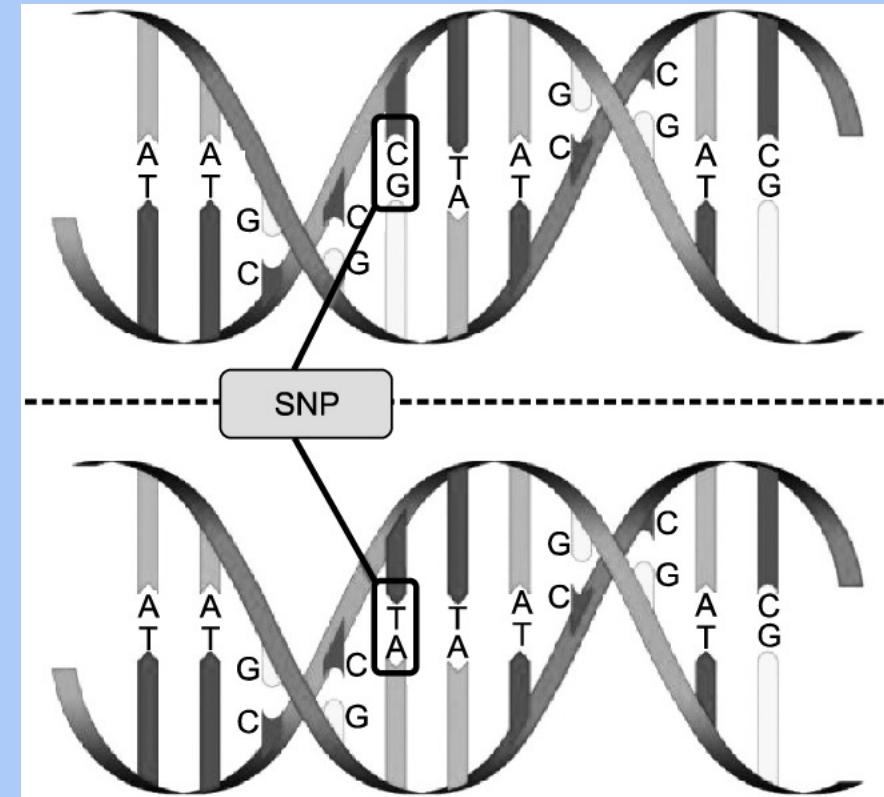
# Single-nucleotide polymorphisms

- Polymorphism is the tendency of DNA to admit of different nucleotide pairs at a single locus



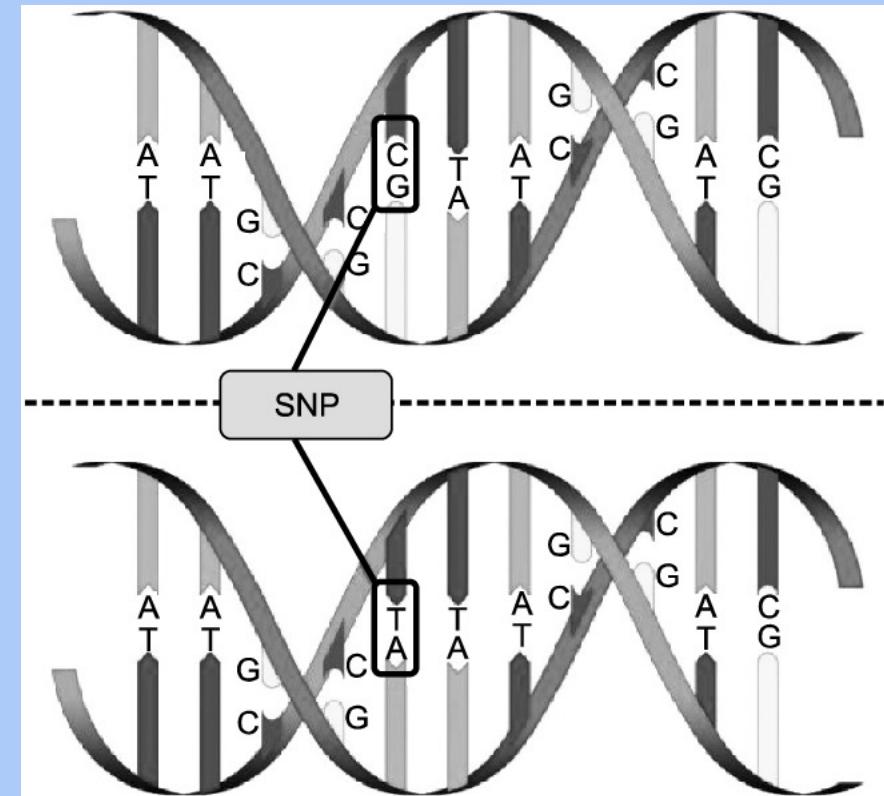
# Single-nucleotide polymorphisms

- Of 3.2 billion bases, any individual is polymorphic at 4-5 million sites



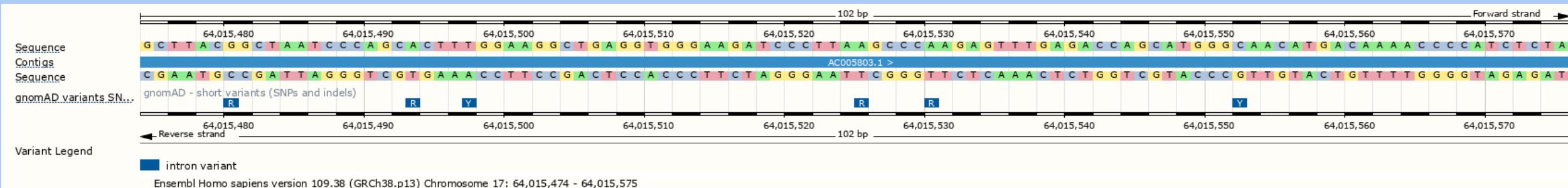
# Single-nucleotide polymorphisms

- The more common allele is called the **major allele**
- The less common allele is called the **minor allele**



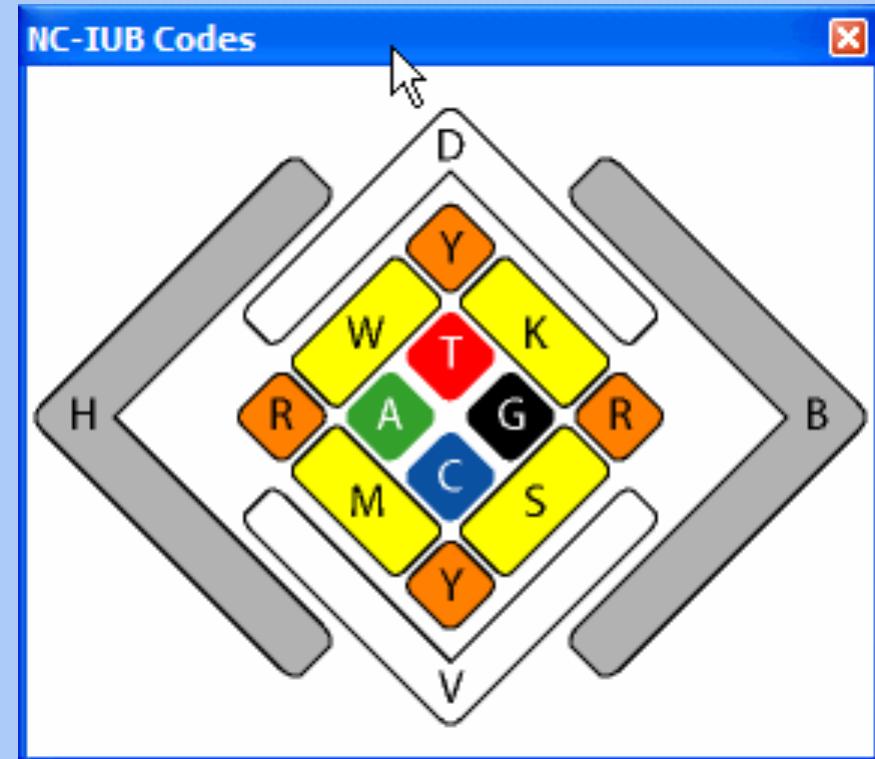
# IUPAC-IUB SNP codes

- More than just A, T, G, and C?



# IUPAC-IUB SNP codes

- Each polymorphism is coded by its possible alleles



[https://www.gendx.com/SBTengine/  
Help\\_220/hs310.htm](https://www.gendx.com/SBTengine/Help_220/hs310.htm)

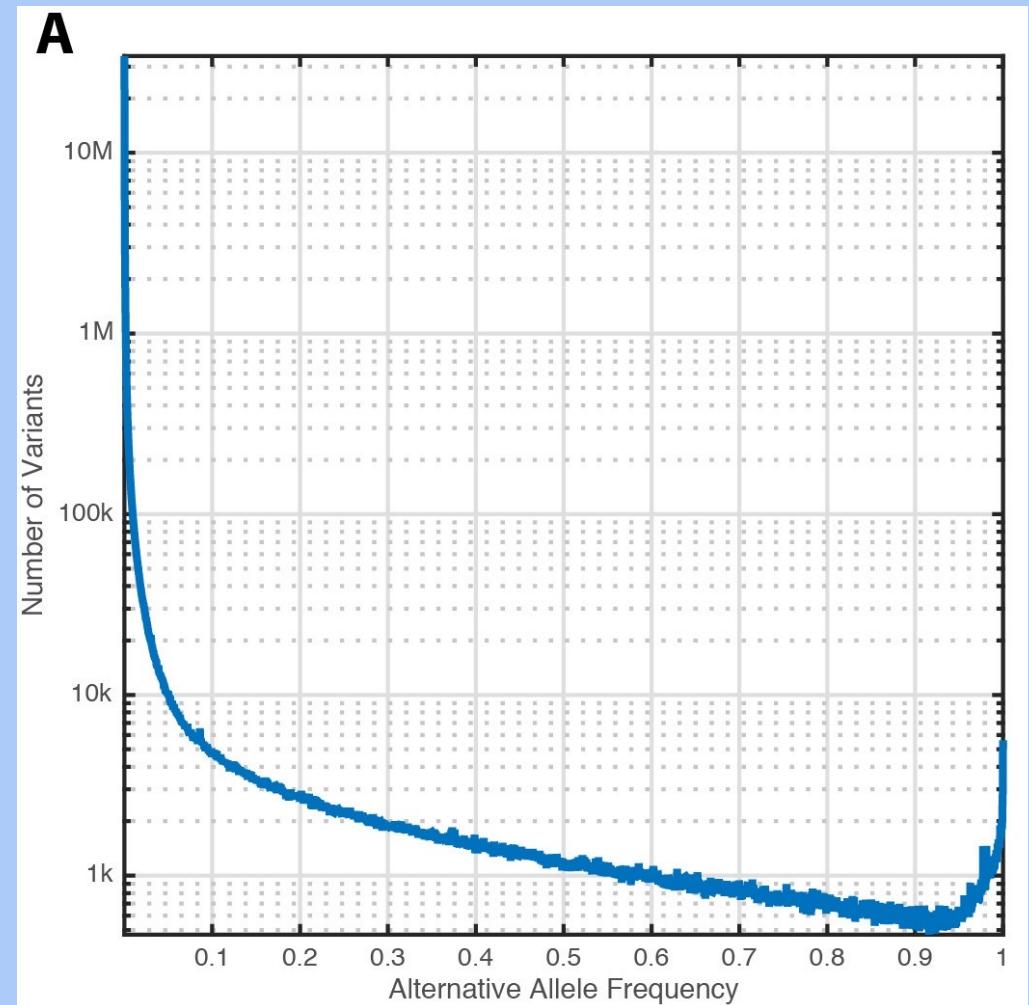
# IUPAC-IUB SNP codes

- Each polymorphism is coded by its possible alleles

Code	Meaning	Explanation
R	A or G	PuRrine
Y	C or T	PYrimidine
S	G or C	Strong H-bonding
W	A or T	Weak H-bonging
K	G or T	Keto bases
M	A or C	aMino bases
B	C or G or T	not A
D	A or G or T	not C
H	A or C or T	not G
V	A or C or G	not T
N	A or C or G or T	ANy

# Many rare SNPs

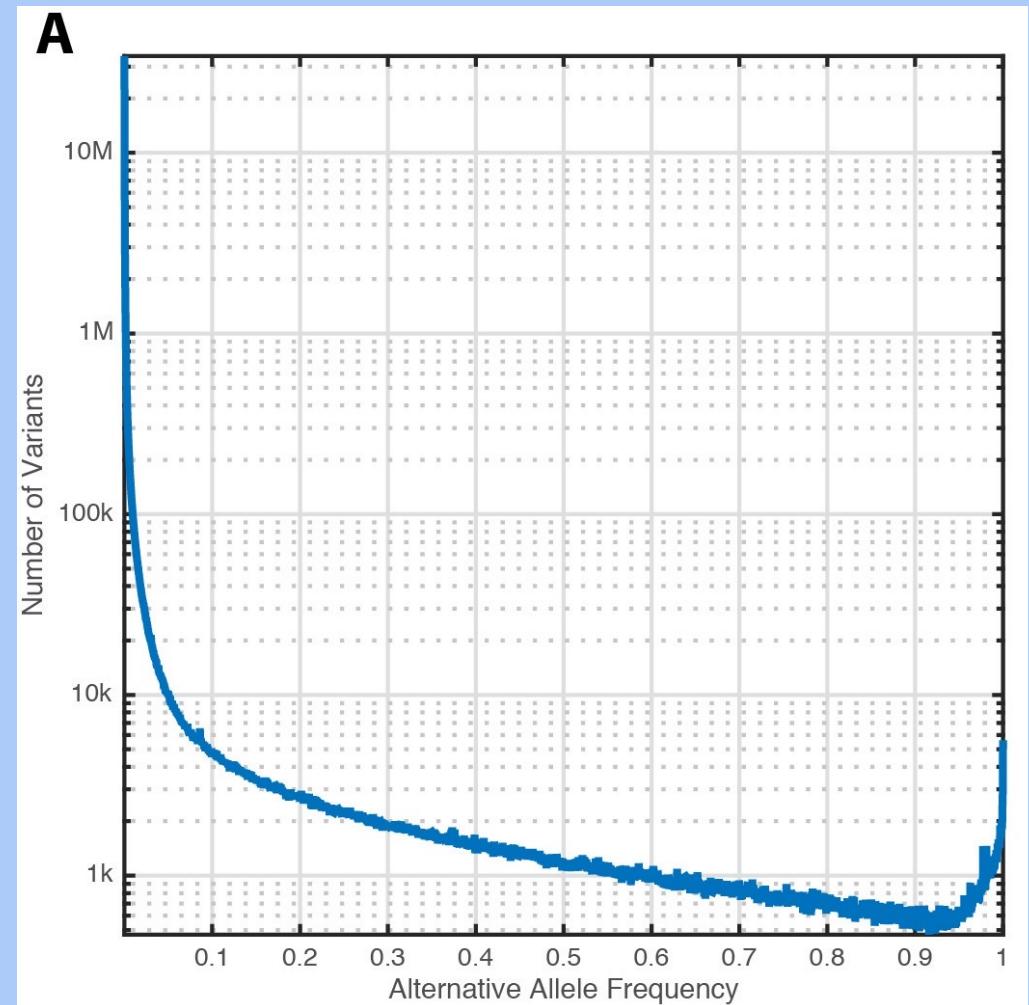
- Common SNPs have minor allele frequency (MAF) >5%



<https://www.nature.com/articles/nature15393>

# Many rare SNPs

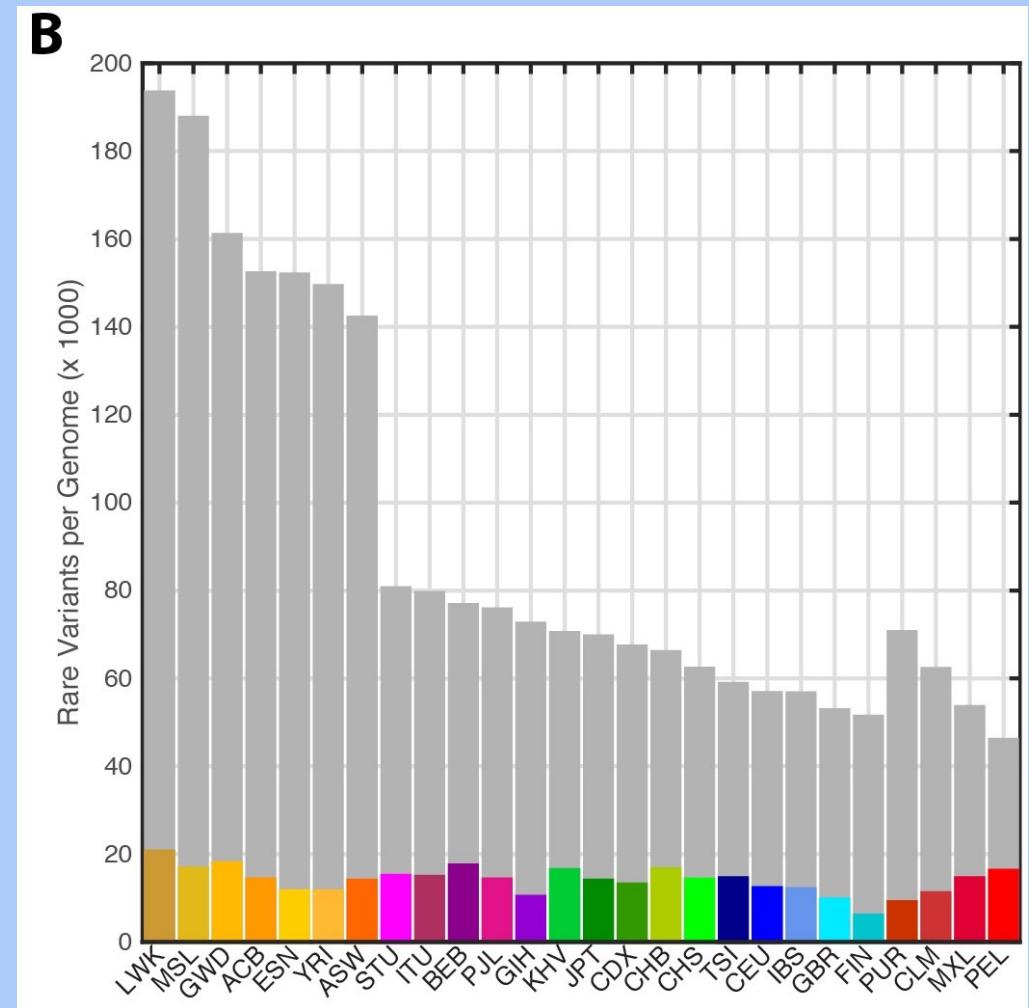
- Most SNPs of the >600 million known SNPs are very rare (frequency < 0.5%)



<https://www.nature.com/articles/nature15393>

# Many rare SNPs

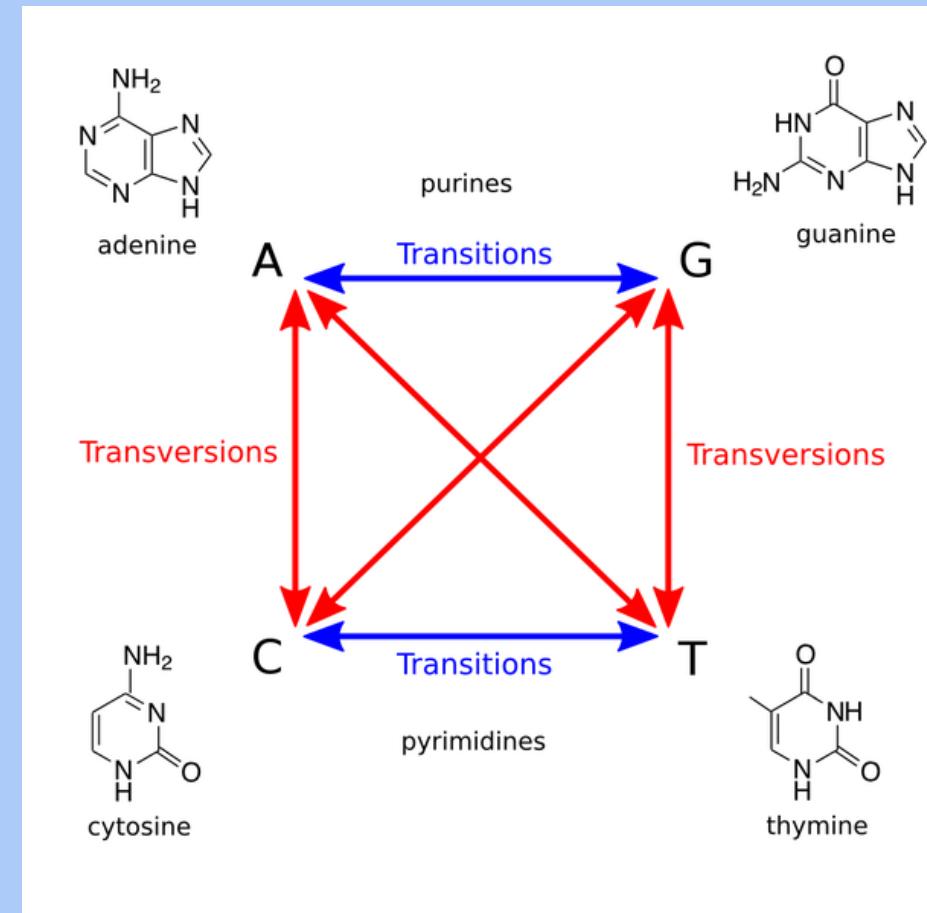
- But only <5% of an individual's genome consists of rare SNPs



<https://www.nature.com/articles/nature15393>

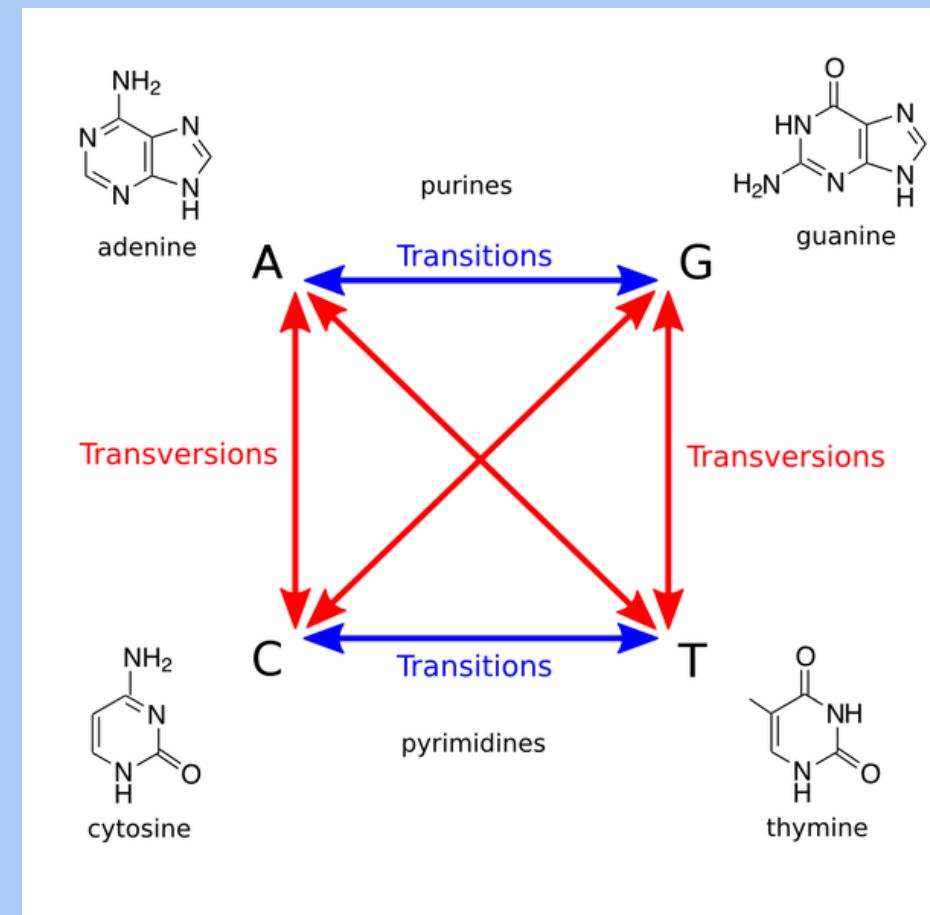
# Transitions and transversions

- **Transitions** occur between nucleotides of the same type (purines or pyrimidines)



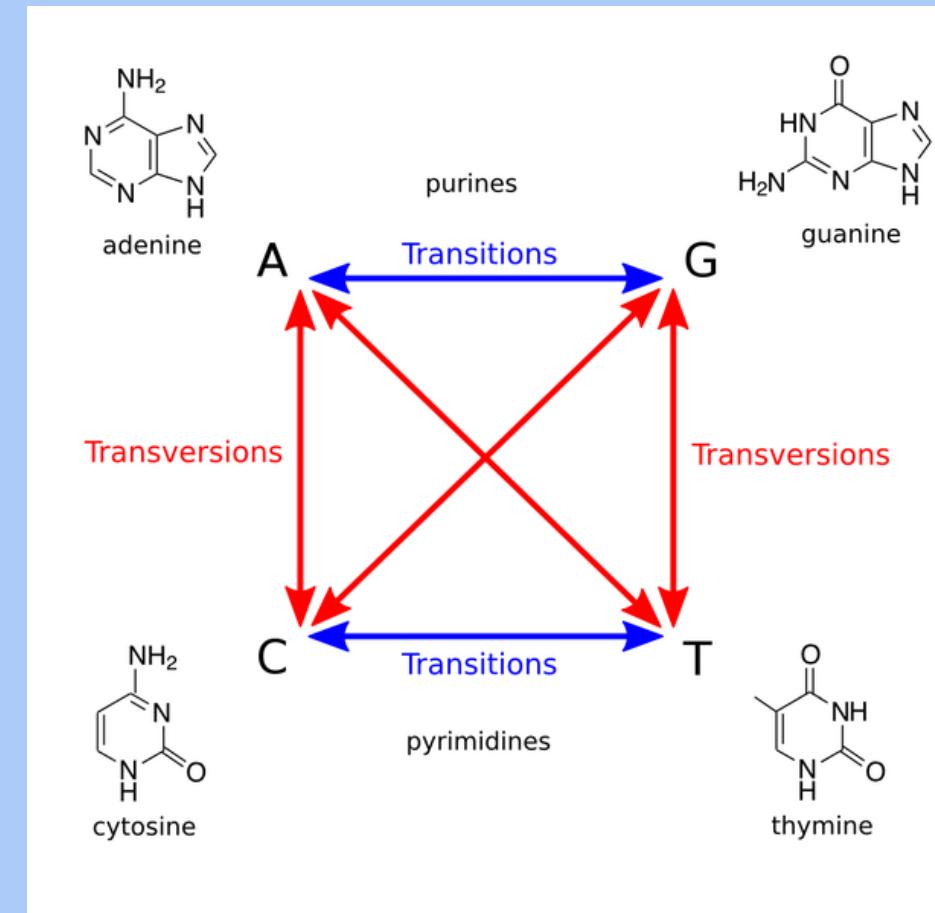
# Transitions and transversions

- Transversions occur between nucleotides of opposite type (between purines and pyrimidine)



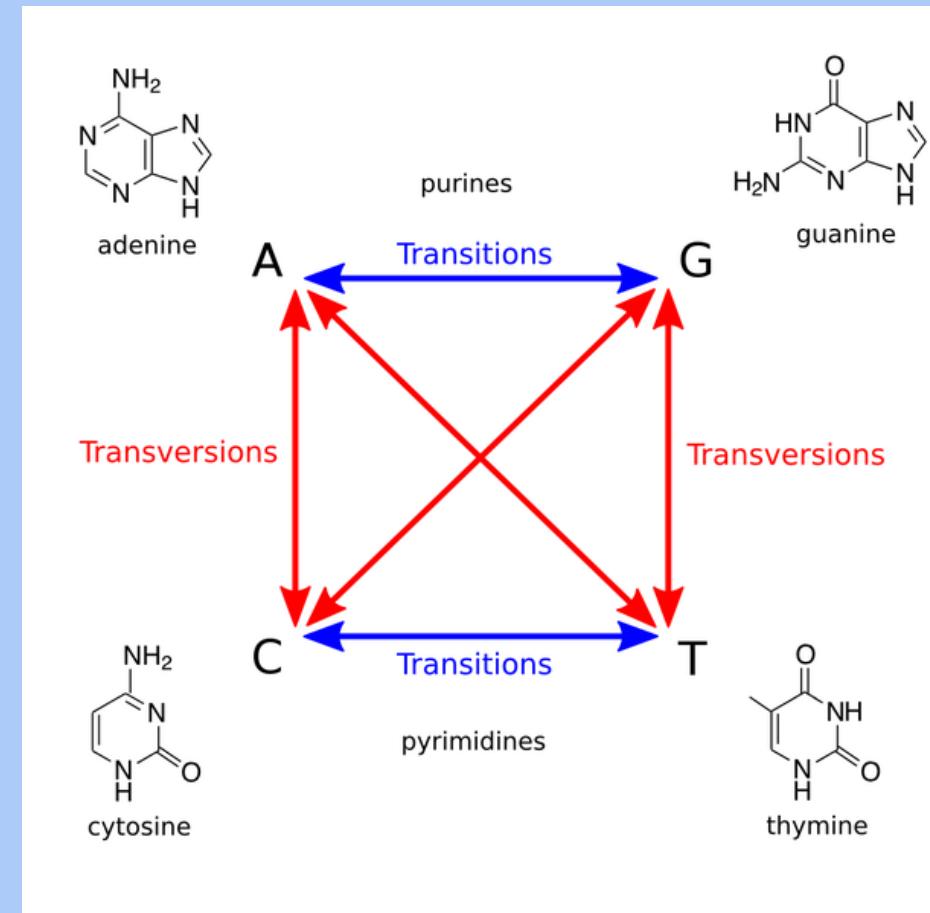
# How many polymorphisms are there?

- If there are  $n$  nucleotide pairs, there are  $n$  symmetric conversions:
  - A/T  $\rightarrow$  T/A transversion
  - C/G  $\rightarrow$  G/C transversion



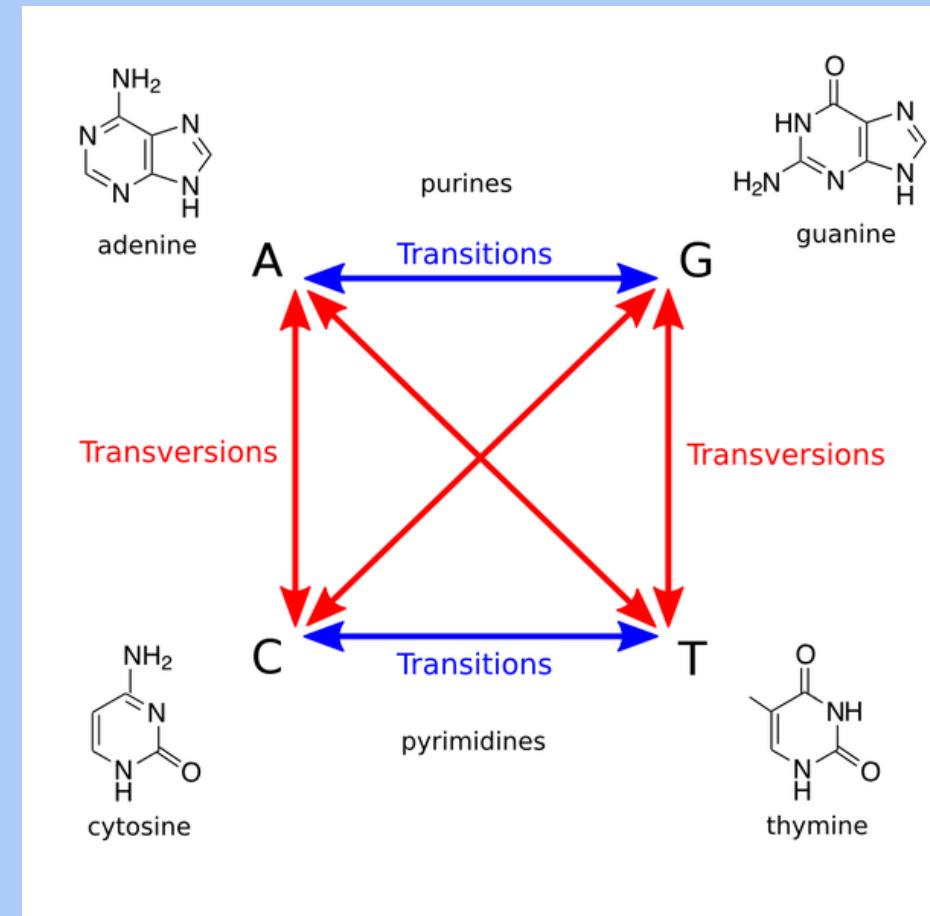
# How many polymorphisms are there?

- If there are  $n$  nucleotide pairs, there are  $n(n - 1)$  **asymmetric** conversions:
  - A/T → C/G transversion
  - A/T → G/C transition



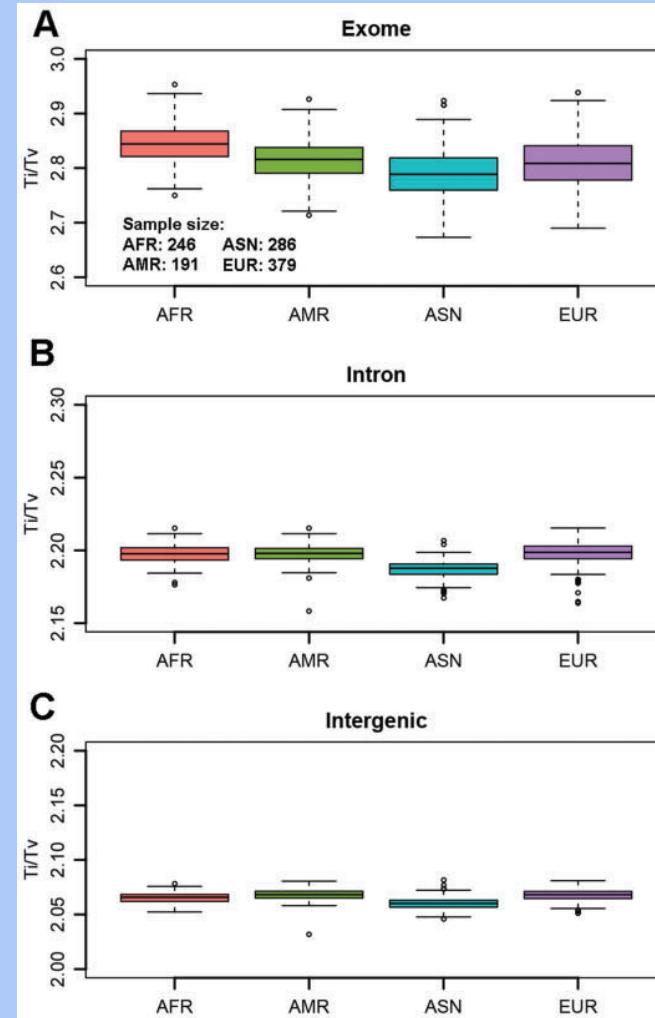
# How many polymorphisms are there?

- A total of  $n + n(n - 1) = n^2$  polymorphisms



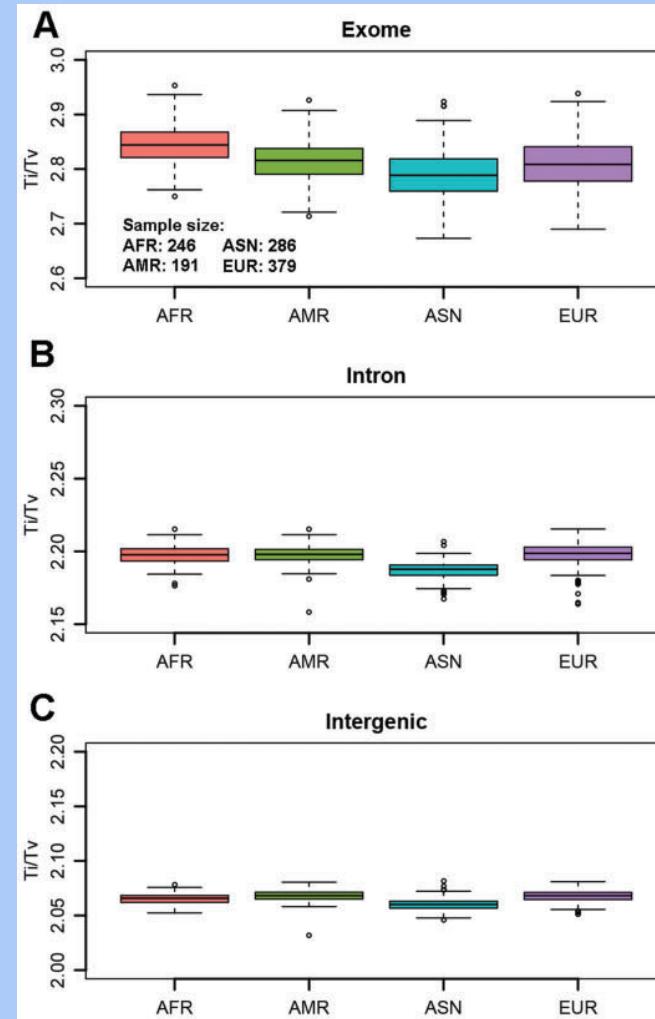
# Transition-transversion ratio

- Even though there are three times as many transversions possible as transitions, in humans the ratio of transitions to transversions is approximately 2, genome-wide



# Transition-transversion ratio

- In coding regions, the Ti:Tv ratio is as high as 3



# Generation of sequencing data

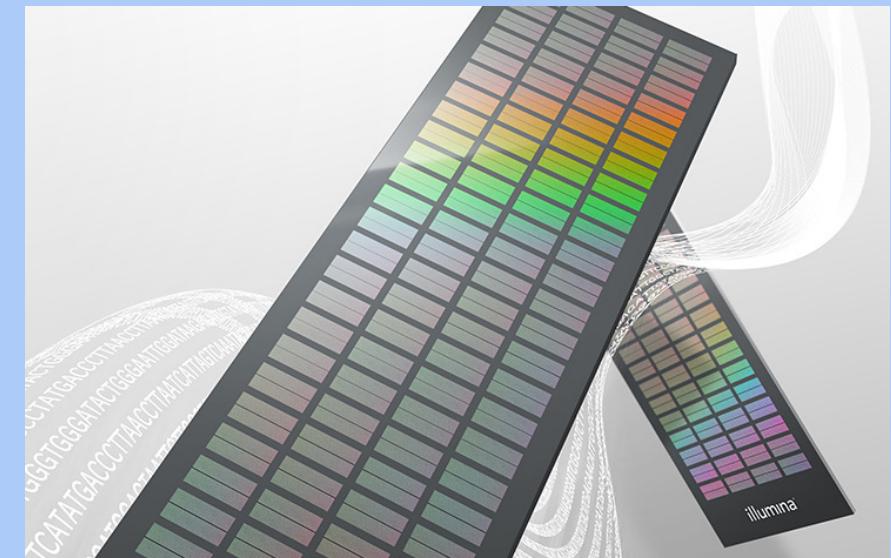
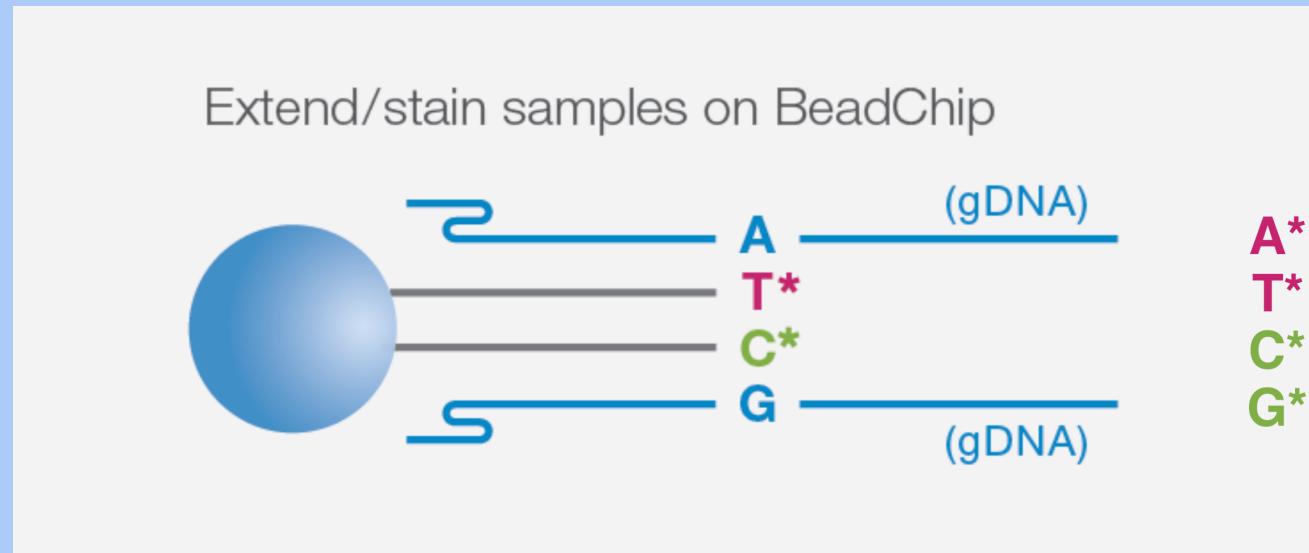
Sequencing technologies and data formats

# How do we get human genotypes?

- SNP Chips
- Whole-genome sequencing

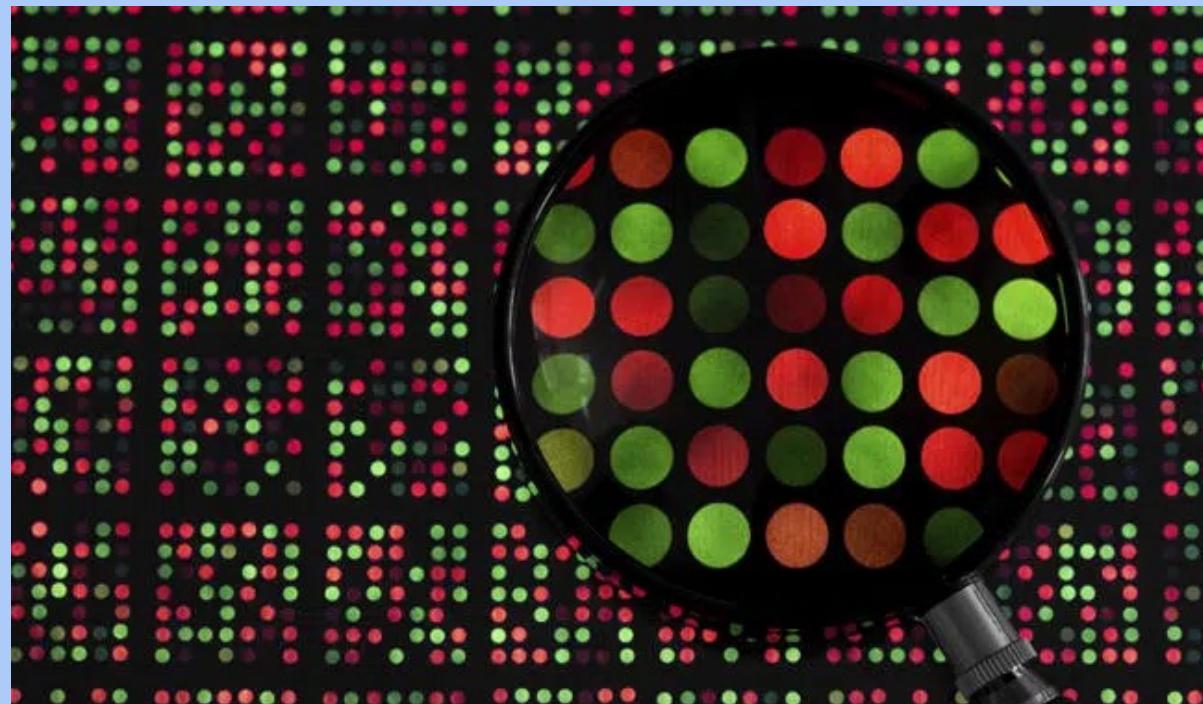
# SNP Chips

- Genomic DNA binds to a complementary sequence and incorporates a fluorescently labelled nucleotide



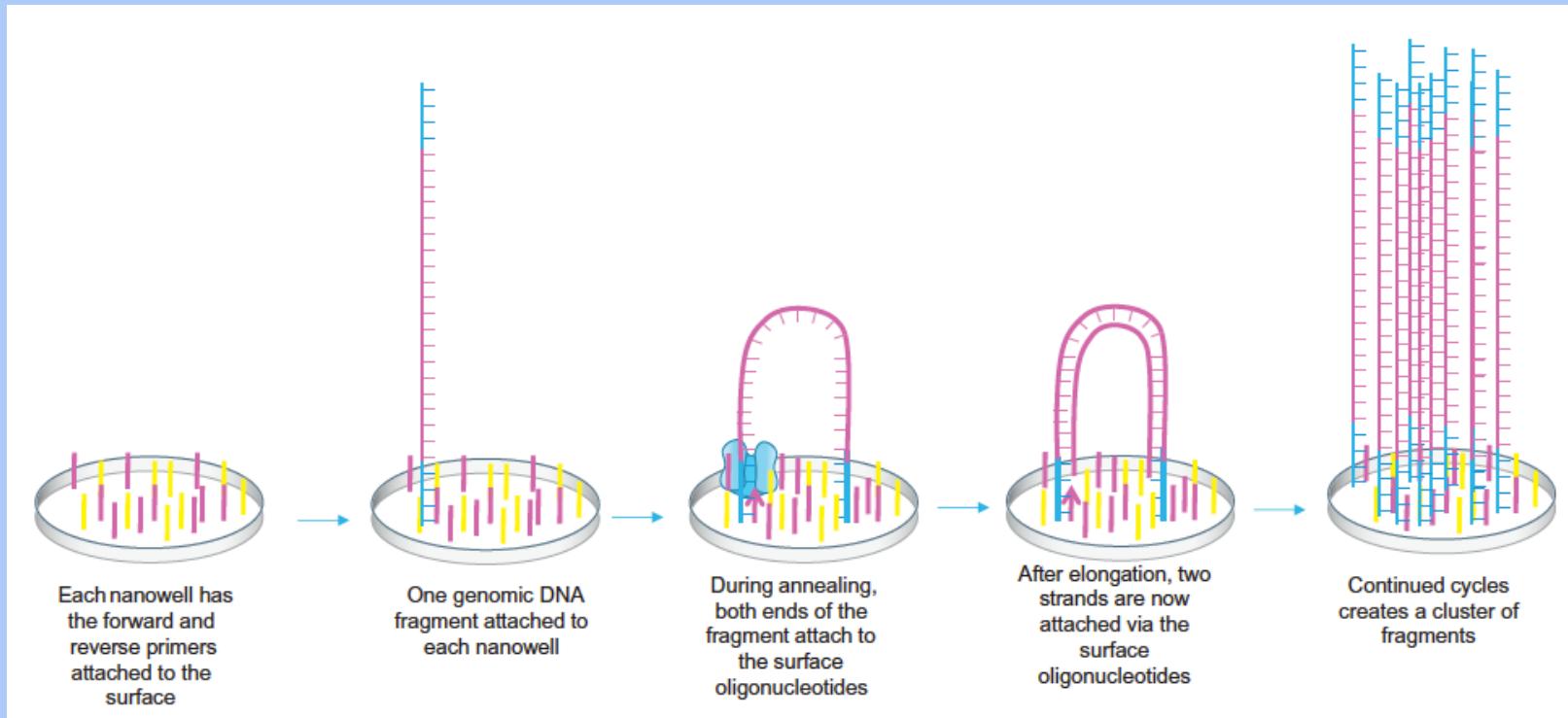
# SNP Chips

- The ratio of red to green at a spot identifies the sample allele



# Whole-genome sequencing (WGS)

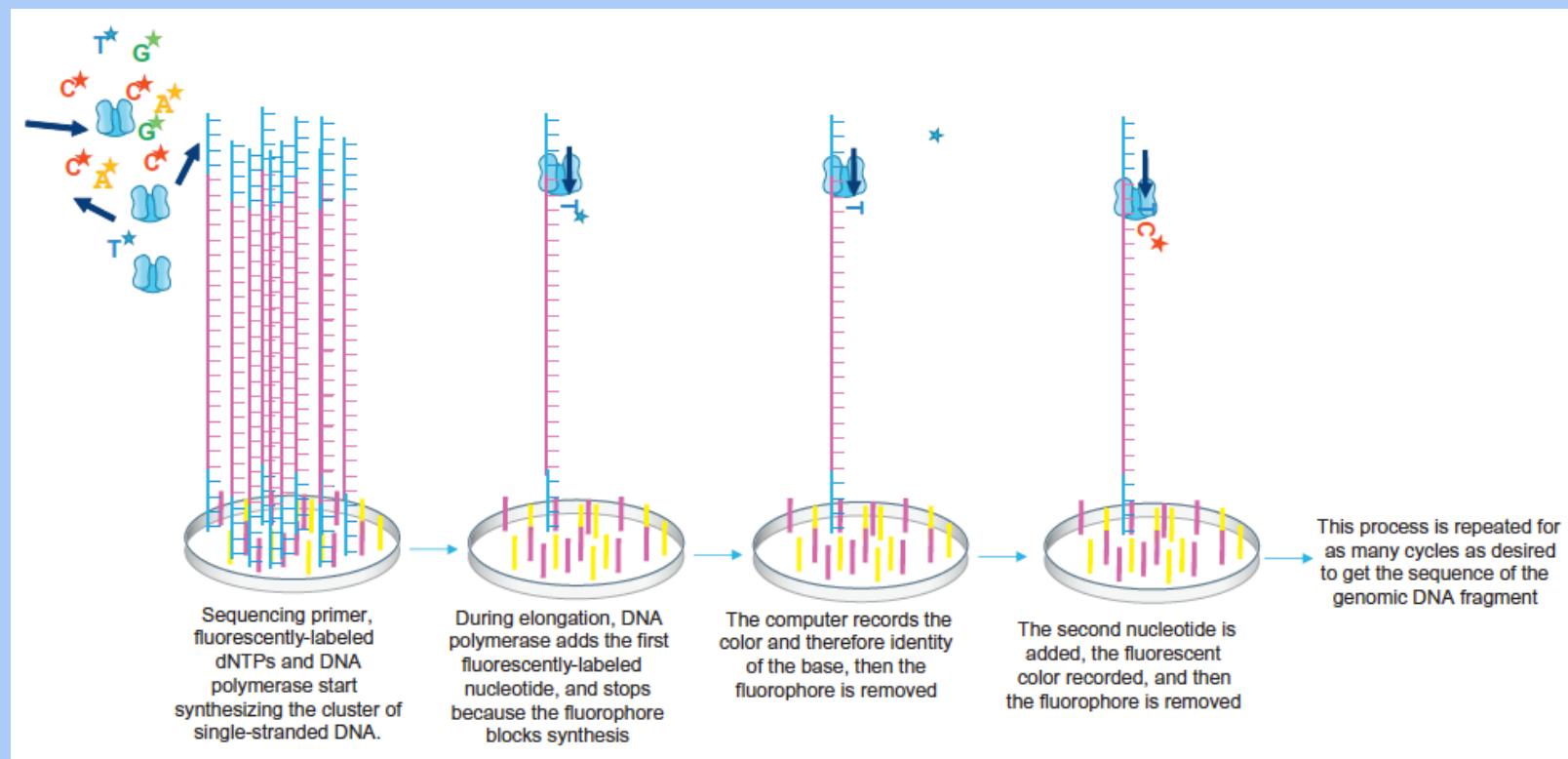
- DNA fragments from a sample are attached to a flow cell and amplified



Clark et al. *Molecular Biology (3<sup>rd</sup> Edition)*. Ch. 8: DNA Sequencing, 240-269 (2019)

# Whole-genome sequencing (WGS)

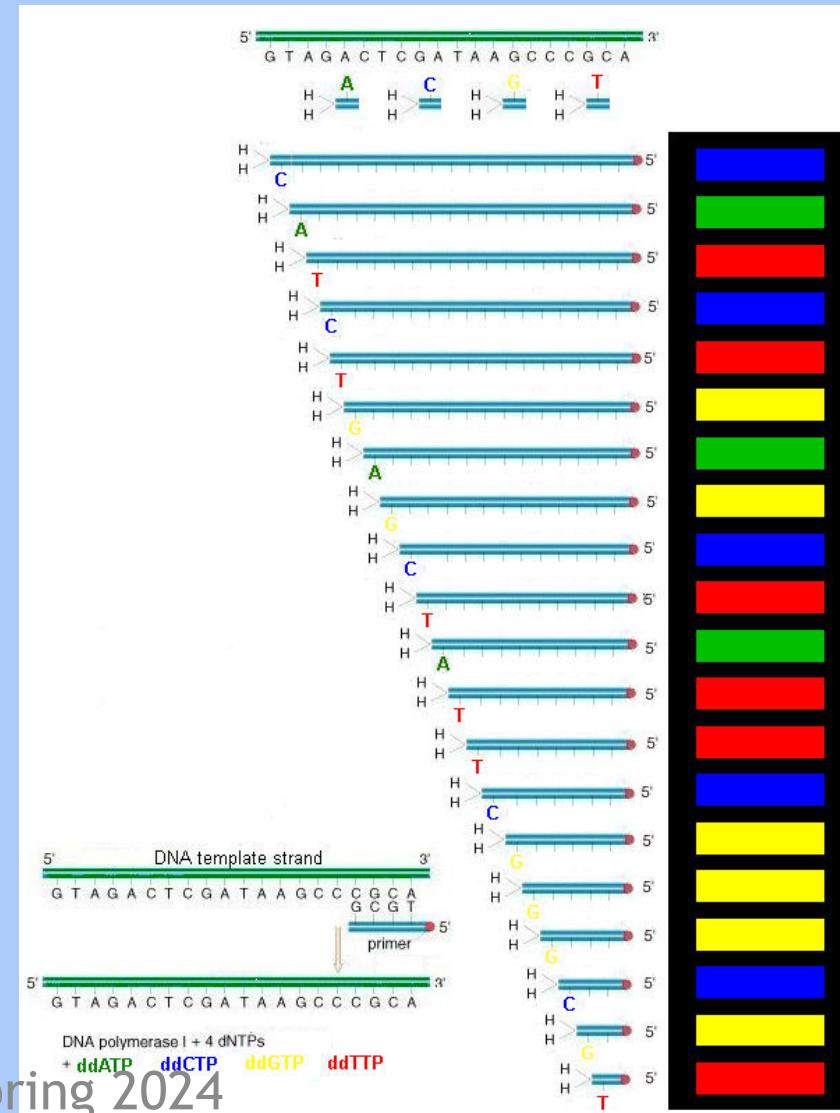
- **Sequencing by synthesis:** Short reads are produced as fluorescent nucleotides are incorporated one base at a time



Clark et al. *Molecular Biology (3<sup>rd</sup> Edition)*. Ch. 8: DNA Sequencing, 240-269 (2019)

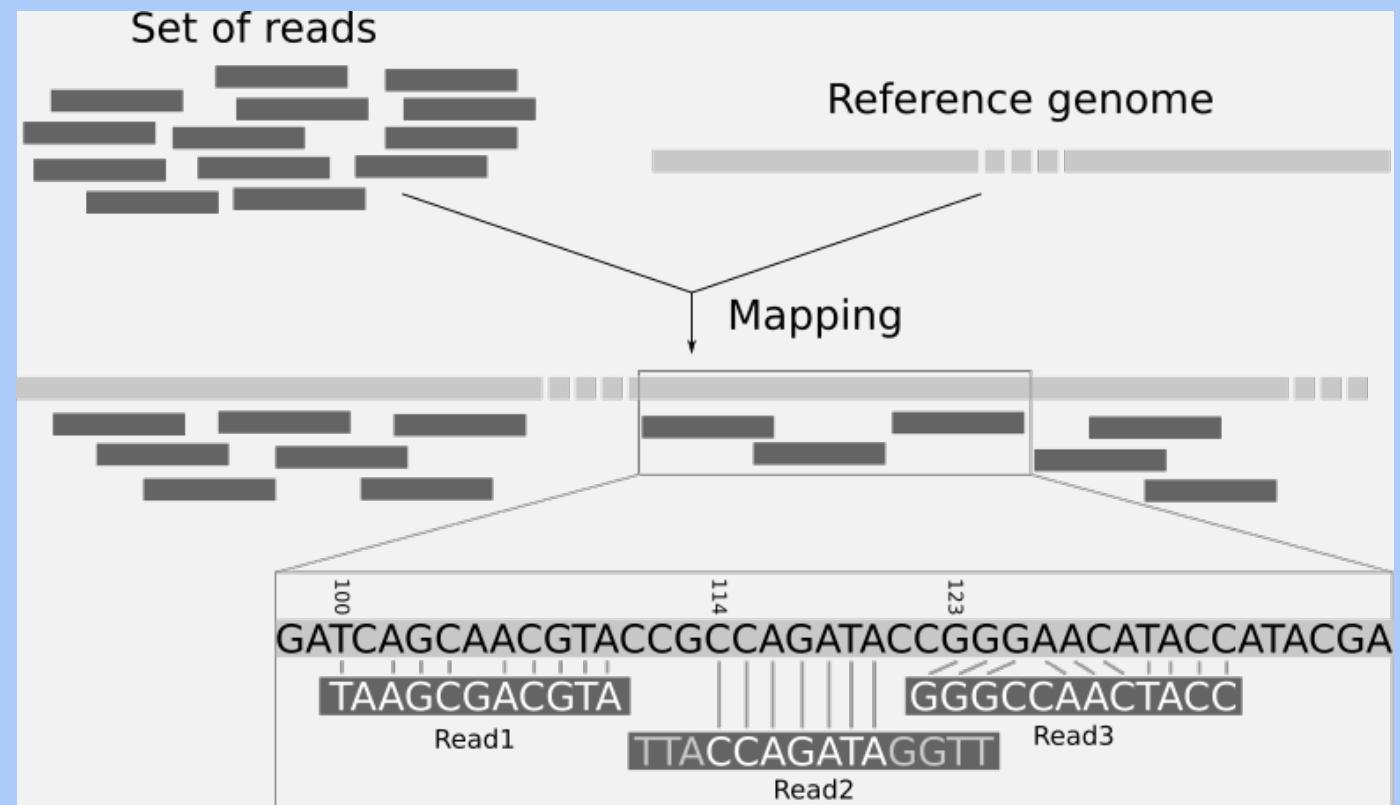
# Whole-genome sequencing (WGS)

- The DNA sequence is inferred from the sequence of fluorescence images



# Mapping to the reference genome

- Locate where in the genome the reads came from, and detect single-nucleotide differences from the reference sequence



# Data-processing pipeline

- Generate raw reads
- Align to a reference genome
- Detect variant sites

# FASTQ

- Contains raw sequence reads and their quality scores to be aligned to a reference genome (FASTA)

```
@A00178:71:HGT77DSXX:1:2171:1:17/0//:80// Z:N:0:ACAGCAAC+GTTGCTGT  
GAAGAAAAGAAGGACACAGAGGAGGGAAAGGTTGAGGAAATTGATGAAGAGAAGGAAGAGAAGAAAAGAAGACGATCAAGGAGGTTT  
+  
FFFFFFFFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFF:FFFFFFFFFFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:1507:30291:23422 1:N:0:ACAGCAAC+GTTGCTGT  
ACATAGAGCTTGTGTTGCCCTCTCCTGGTGTCAAAGGGGGCCTTGGGACAAAAGGACAGCCTGAACCTCAAGCT  
+  
FFFFFFFFFFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:1507:30291:23422 2:N:0:ACAGCAAC+GTTGCTGT  
CTGGATGAGGAAGCCTGAGGAGATACCAAGGAGGTATGCTGCTTCTATAAAAGCTTGACAAATGACTGGGAAGAGCATCTGGCTGTCAAG  
+  
FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:2413:22806:35790 1:N:0:ACAGCAAC+GTTGCTGT  
GCTTGATGTTGCCCTCTCCTGGTGTCAAAGGGGGCCTTGGGACAAAAGGACAGCCTGAACCTCAAGCTGCCCTC  
+  
FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:2413:22806:35790 2:N:0:ACAGCAAC+GTTGCTGT  
GAGAAGAAAAGAACGATCAAGGAGGTTCTCATGAATGGTCCTGATCAACAAGCAGAACCTATCTGGATGAGGAAGCCTGAGGAGATCA  
+  
F:FF:FFFFFFFFFF,:FFFFFFFFFF:FFFFFFFFFF:F:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:2354:5620:8876 1:N:0:ACAGCAAC+GTTGCTGT  
ATGTTGTTGCCCTCTCCTGGTGTCAAAGGGGGCCTTGGGACAAAAGGACAGCCTGAACCTCAAGCTGCCCTCTACAG/  
+  
FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:2354:5620:8876 2:N:0:ACAGCAAC+GTTGCTGT  
AGAAGGAAGAGAAAGAGAAAAGAACGATCAAGGAGGTTCTCATGAATGGTCCTGATCAACAAGCAGAACCTATCTGGATGAGGAAC  
+  
FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF  
@A00178:71:HGT77DSXX:1:1560:6741:9815 1:N:0:ACAGCAAC+GTTGCTGT  
GCAGGATTTACCATGACTACTTTGTATGCCAGAGAAGCTAGATTTGCCAATGATGTTATAGACCATTACGTTGCCAAGC  
+  
FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF:FFFFFFFFFF
```

# SAM (BAM)

- Paired-end reads are aligned to either the forward or reverse strand of the reference genome

```
5' ACATAGACAGGGACCACCTGCAGGACACACACCGCAGGTTACTAAGGGTTACTCAACACAGTGAACAGCATATACCAGA 3'
```

```
5' ACCTGCAGGACACACACCGCAGGTTACTAAGGGTTACTCAACACAGTGA 3'
```

```
|||||||||||||||||||||||||||||||||||||||
```

```
3' TGGACGTCCCTGTGTGCGTCAAATGATTCCAAATGAGTTGTGTCACT 5'
```

<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# SAM (BAM)

- Paired-end reads are aligned to either the forward or reverse strand of the reference genome

```
Read 1: 5' ACCTGCAGGA 3'  
5' ACATAGACAGGGACCACCTGCAGGACACACACCGCAGGTTACTAAGGGTTACTCAACACAGTGAACAGCATATACAGA 3'  
forward-strand  
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
reverse-strand  
3' TGTATCTGCCCTGGTGGACGTCCCTGTGTGCGTCAAATGATTCCAAATGAGTTGTGTCATTGTCGTATATGGTCT 5'  
Read 2: 3' TTGTGTCACT 5'
```

<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# SAM (BAM)

- Paired-end reads are aligned to either the forward or reverse strand of the reference genome

```
Read 2: 5' ACCTGCAGGA 3'  
5' ACATAGACAGGGACCACCTGCAGGACACACACCGCAGGTTACTAAGGGTTACTCAACACAGTGAACAGCATATACCAGA 3'  
forward-strand  
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||  
reverse-strand  
3' TGTATCTGTCCCTGGTGGACGTCCCTGTGTGCGTCAAATGATTCCAAATGAGTTGTGTCATTGTCGTATATGGTCT 5'  
Read 1: 3' TTGTGTCACT 5'
```

<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# SAM (BAM)

- A Sequence alignment map (SAM) or binary alignment map (BAM) file contains the alignments to the reference genome

**A**

Coor	10	20	30	40
ref	12345678901234	5678901234567890123456789012345		
	AGCATTTAGATAAA**GATAGCTGTGCTAGTAGGCAGTCAGCGCCAT			
+r001/1		TTAGATAAAGGATA*CTG		
+r002		aaaAGATAA*GGATA		
+r003		gcctaAGCTAA		
+r004		ATAGCT.....TCAGC		
-r003			ttagctTAGGC	
-r001/2				CAGCGGCAT

**B**

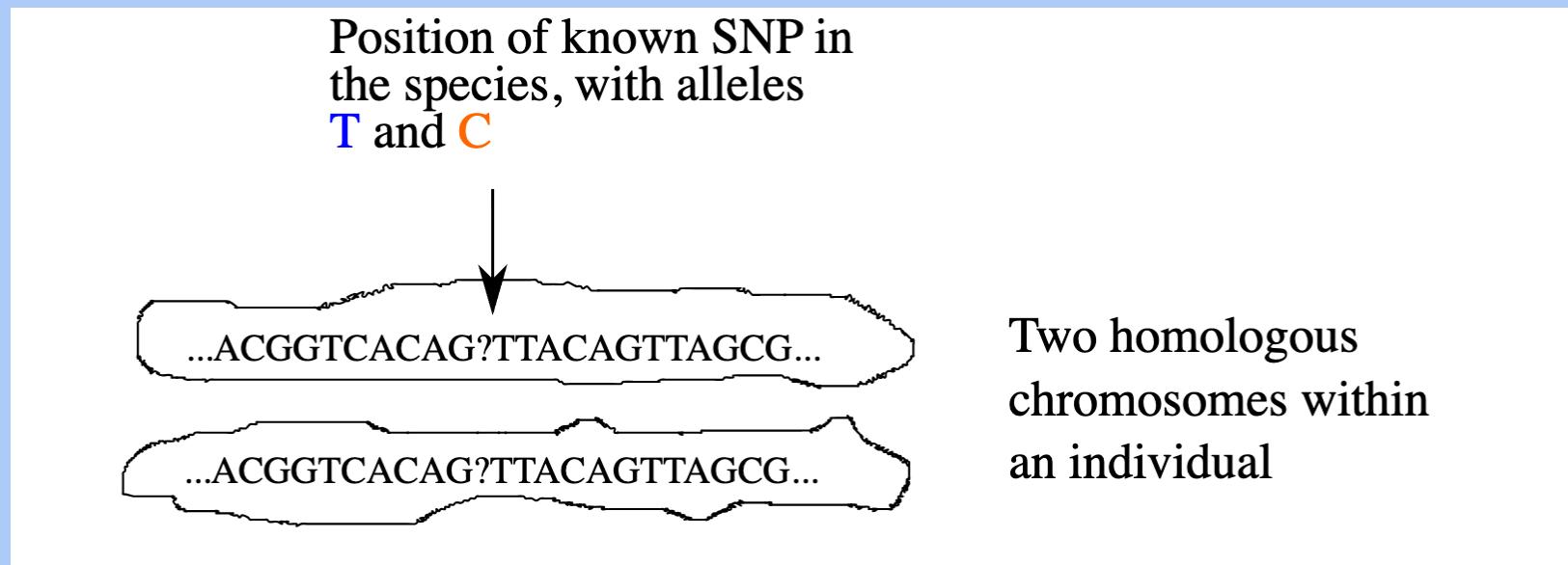
Header section		QUAL (read quality; * meaning such information is not available)	
@HD VN:1.5 SO:coordinate			
@SQ SN:ref LN:45			
r001	99 ref 7 30 8M2I4M1D3M = 37 39 TTAGATAAAGGATACTG *		
r002	0 ref 9 30 3S6M1P1I4M * 0 0 AAAAGATAAGGATA *		
r003	0 ref 9 30 5S6M * 0 0 GCCTAAGCTAA * SA:Z:ref,29,-,6H5M,17,0;		
r004	0 ref 16 30 6M14N5M * 0 0 ATAGCTTCAGC *		
r003	2064 ref 29 17 6H5M * 0 0 TAGGC * SA:Z:ref,9,+,5S6M,30,1;		
r001	147 ref 37 30 9M = 7 -39 CAGCGGCAT * NM:i:1		

Annotations below the table:

- QNAME FLAG (query template name, aka. read ID)
- RNAME (reference sequence name, e.g. chromosome /transcript id)
- POS (1-based position)
- MAPQ (mapping quality)
- CIGAR (summary of alignment, e.g. insertion, deletion)
- RNEXT (reference sequence name of the primary alignment of the NEXT read; for paired-end sequencing, NEXT read is the paired read; corresponding to the RNAME column)
- PNEXT (Position of the primary alignment of the NEXT read in the template; corresponding to the POS column)
- TLEN (the number of bases covered by the reads from the same fragment. In this particular case, it's 45 - 7 + 1 = 39 as highlighted in Panel A). Sign: plus for leftmost read, and minus for rightmost read
- SEQ (read sequence)
- Optional fields in the format of TAG:TYPE:VALUE

# Variant calling (mpileup)

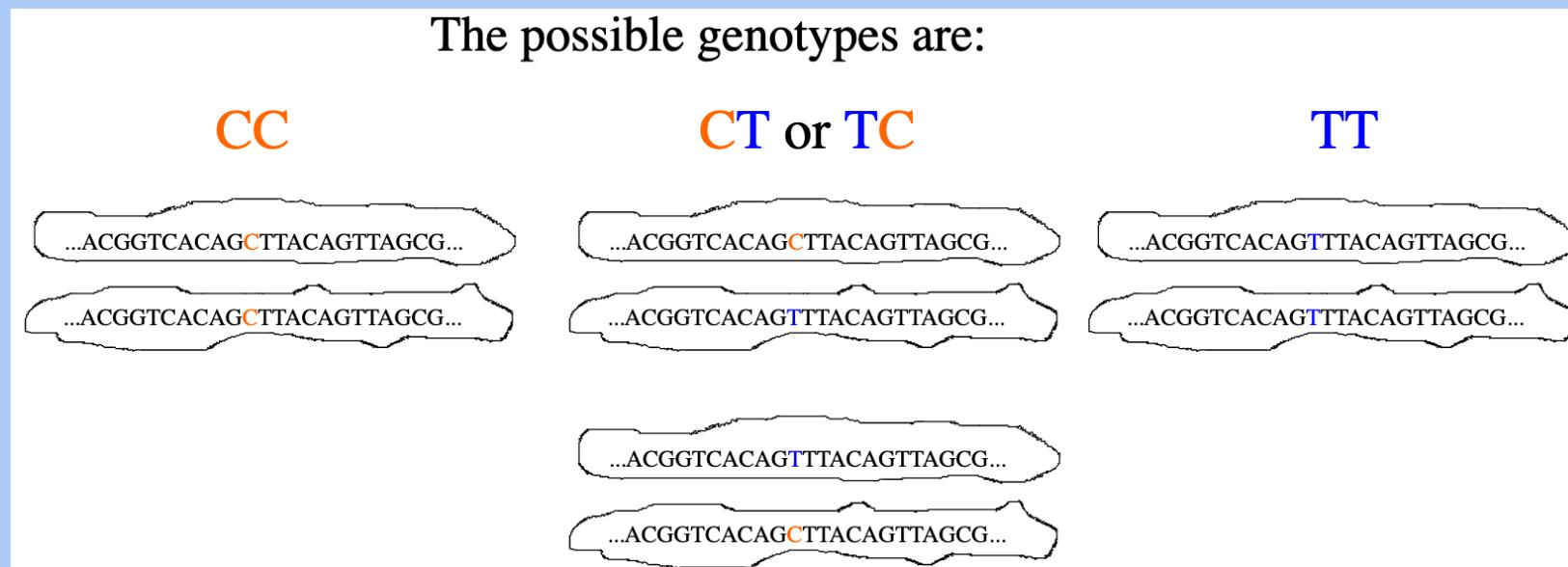
- How certain can we be of an individual's genotype?



<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# Variant calling (mpileup)

- How certain can we be of an individual's genotype?



<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# Variant calling (mpileup)

- How certain can we be of an individual's genotype?

The data are: 4 reads covering that site,  
*and*  
the associated base quality scores

<i>Read</i>			<i>Observed Base</i>	<i>PHRED-scaled base quality score</i>
#	<i>Read</i>			
1	CAG <b>C</b> TTACA		<b>C</b>	32 (A)
2	ACAG <b>C</b> T		<b>C</b>	37 (F)
3	<b>G</b> TTTA		<b>T</b>	35 (D)
4	AG <b>C</b> TTACAG		<b>C</b>	33 (B)

<https://eriqande.github.io/eca-bioinf-handbook/bioinformatic-file-formats.html#sambamfiles>

# VCF

- The results of genotype-calling are stored in a variant call format (VCF) file

VCF															
#fileformat=VCFv4.2 ##contig=<ID=2,length=51304566> ##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes"> ##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes"> ##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype"> ##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth"> ##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality"> #CHROM POS ID REF ALT QUAL FILTER INFO FORMAT SAMPLE1 SAMPLE2 SAMPLE3 SAMPLE4 SAMPLE5 SAMPLE6 SAMPLE7															
2	81170	.	C	T	.	.	AC=9;AN=7424	GT:DP:GQ	0/0:4:12	0/0:3:9	0/1:1:3	0/1:9:24	1/0:4:12	0/0:5:15	0/0:4:12
2	81171	.	G	A	.	.	AC=6;AN=7446	GT:DP:GQ	0/1:4:12	0/0:3:9	0/0:1:3	0/0:9:24	0/1:4:12	0/1:5:15	0/0:4:12
2	81182	.	A	G	.	.	AC=5;AN=7506	GT:DP:GQ	0/0:5:15	0/0:4:12	0/0:5:15	0/0:9:24	0/0:4:12	0/0:4:12	0/0:4:12
2	81204	.	T	G	.	.	AC=2;AN=7542	GT:DP:GQ	1/0:5:15	0/0:9:27	0/0:10:30	0/0:15:39	0/0:9:27	1/0:13:39	0/1:14:42

BCF										
2	81170	.	C	T	.	.	AC=9;AN=7424	GT:0/0:0/0:0/0:1/0:1/0:0/0:0/0:0/0	DP:4:3:1:9:4:5:4	GQ:12: 9: 3:24:12:15:12
2	81171	.	G	A	.	.	AC=6;AN=7446	GT:0/1:0/0:0/0:0/0:0/1:0/1:0/0	DP:4:3:1:9:4:5:4	GQ:12: 9: 3:24:12:15:12
2	81182	.	A	G	.	.	AC=5;AN=7506	GT:0/0:0/0:0/0:0/0:0/0:0/0:0/0	DP:5:4:5:9:4:4:4	GQ:15:12:15:24:12:12:12
2	81204	.	T	G	.	.	AC=2;AN=7542	GT:1/0:0/0:0/0:0/0:0/0:1/0:0/1	DP:5:9:10:15:9:13:14	GQ:15:27:30:39:27:39:42



# VCF

- The VCF file has one row for each variant, and one column for each sequenced individual

##fileformat=VCFv4.0 ##fileDate=20110705 ##reference=1000GenomesPilot-NCBI37 ##phasing=partial ##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data"> ##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth"> ##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency"> ##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele"> ##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129"> ##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership"> ##FILTER=<ID=q10,Description="Quality below 10"> ##FILTER=<ID=s50,Description="Less than 50% of samples have data"> ##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality"> ##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype"> ##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth"> ##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">											
#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	Sample1	Sample2	Sample3
2	4370	rs6857	G	A	29	.	NS=2;DP=13;AF=0.5;DB:H2	GT:GQ:DP:HQ	0 0:48:1:52,51	1 0:48:8:51,51	1/1:43:5:..,
2	7330	.	T	A	3	q10	NS=5;DP=12;AF=0.017	GT:GQ:DP:HQ	0 0:46:3:58,50	0 1:3:5:65,3	0/0:41:3
2	110696	rs6855	A	G,T	67	PASS	NS=2;DP=10;AF=0.333,0.667;AA=T;DB	GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2	2/2:35:4
2	130237	.	T	.	47	.	NS=2;DP=16;AA=T	GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:56,51	0/0:61:2
2	134567	microsat1	GTCT	G,GTACT	50	PASS	NS=2;DP=9;AA=G	GT:GQ:DP	0/1:35:4	0/2:17:2	1/1:40:3

# VCF

- Codes such as GT, DP, GP give the genotype, read depth, and genotype probabilities for each individual

##fileformat=VCFv4.0							FORMAT				
##fileDate=20110705							Sample1	Sample2	Sample3		
##reference=1000GenomesPilot-NCBI37							GT:GQ:DP:HQ	0 0:48:1:52,51	1 0:48:8:51,51	1/1:43:5:,,,,	
##phasing=partial							GT:GQ:DP:HQ	0 0:46:3:58,50	0 1:3:5:65,3	0/0:41:3	
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">							GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2	2/2:35:4	
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">							GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:56,51	0/0:61:2	
##INFO=<ID=AF,Number=.,Type=Float,Description="Allele Frequency">							GT:GQ:DP	0/1:35:4	0/2:17:2	1/1:40:3	
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">											
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">											
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">											
##FILTER=<ID=q10,Description="Quality below 10">											
##FILTER=<ID=s50,Description="Less than 50% of samples have data">											
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">											
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">											
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">											
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">											
CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO				
2	4370	rs6857	G	A	29	.	NS=2;DP=13;AF=0.5;DB:H2	GT:GQ:DP:HQ	0 0:48:1:52,51	1 0:48:8:51,51	1/1:43:5:,,,,
2	7330	.	T	A	3	q10	NS=5;DP=12;AF=0.017	GT:GQ:DP:HQ	0 0:46:3:58,50	0 1:3:5:65,3	0/0:41:3
2	110696	rs6855	A	G,T	67	PASS	NS=2;DP=10;AF=0.333,0.667;AA=T;DB	GT:GQ:DP:HQ	1 2:21:6:23,27	2 1:2:0:18,2	2/2:35:4
2	130237	.	T	.	47	.	NS=2;DP=16;AA=T	GT:GQ:DP:HQ	0 0:54:7:56,60	0 0:48:4:56,51	0/0:61:2
2	134567	microsat1	GTCT	G,GTACT	50	PASS	NS=2;DP=9;AA=G	GT:GQ:DP	0/1:35:4	0/2:17:2	1/1:40:3

# Human genetic variation

Sequencing projects and implications for  
association studies

# The HapMap Project

- International genotyping consortium launched in 2002 to find common polymorphisms linked to rare disease loci



<https://pubmed.ncbi.nlm.nih.gov/16255080/>

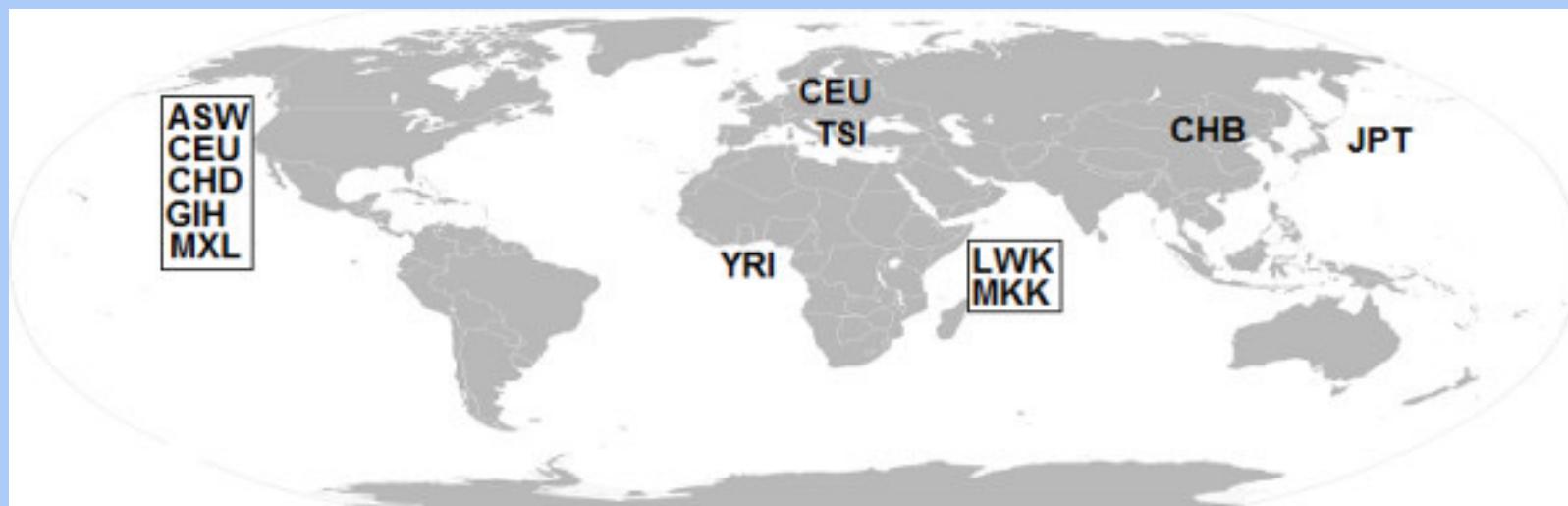
# The HapMap Project

- Variants occur together on a small number of haplotypes



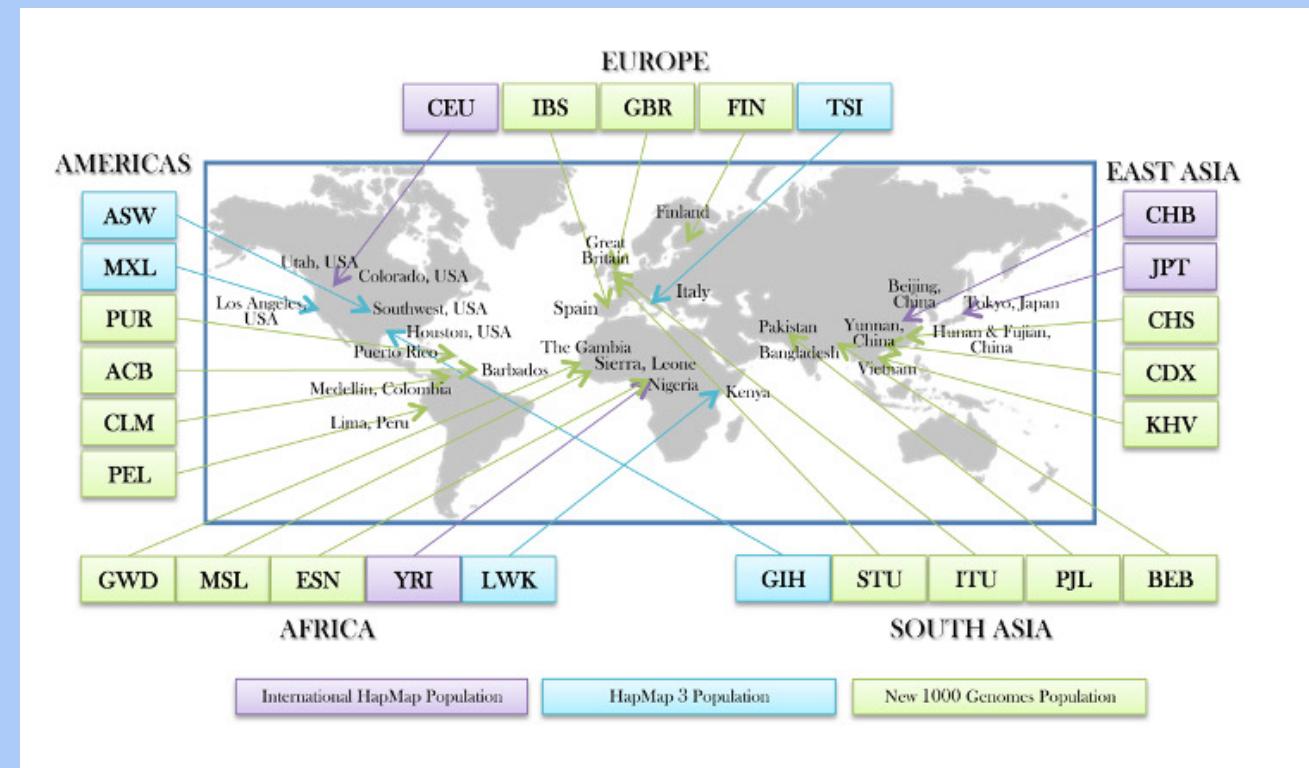
# The HapMap Project

- Phase 3 (2010): genotyping and PCR resequencing of 1.6 million SNPs from 1,184 human samples from different parts of the world



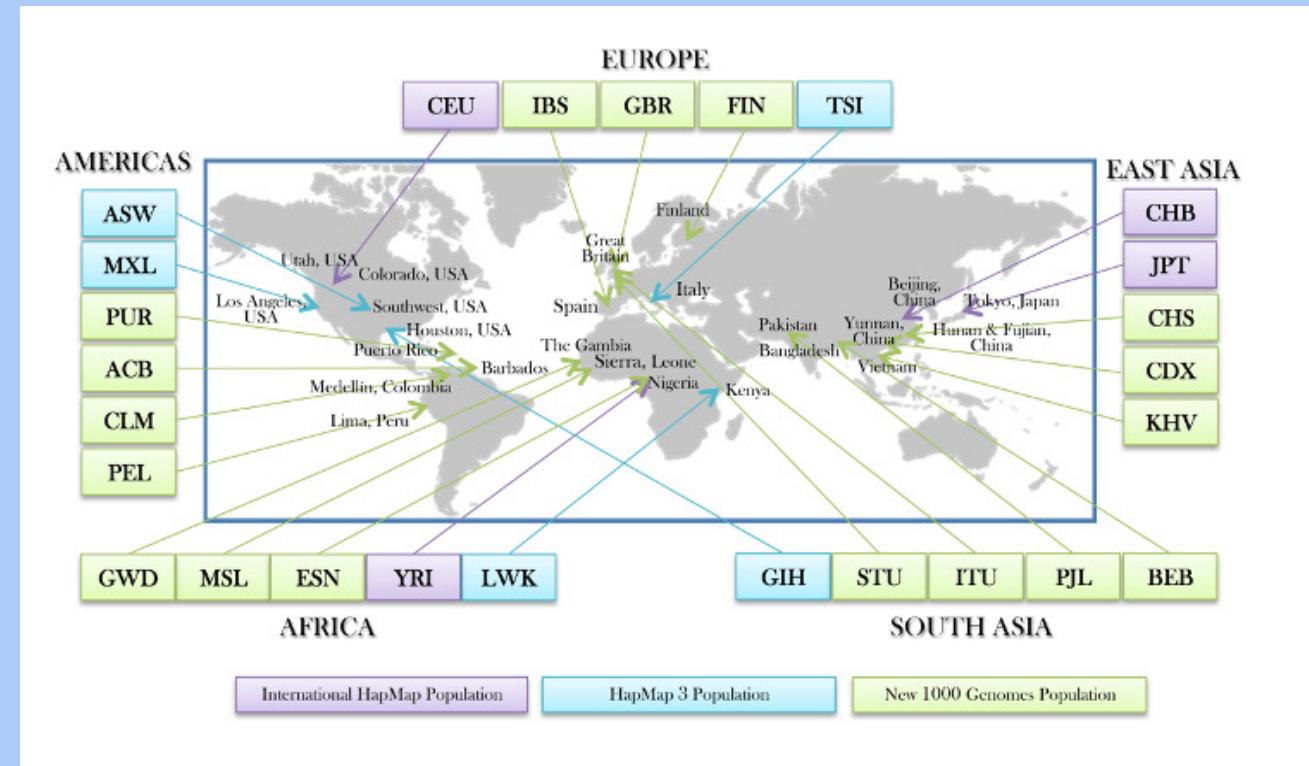
# The 1000 Genomes Project

- An international consortium launched in 2008 to catalog rare variants (frequency < 1%) taking advantage of new sequencing technologies

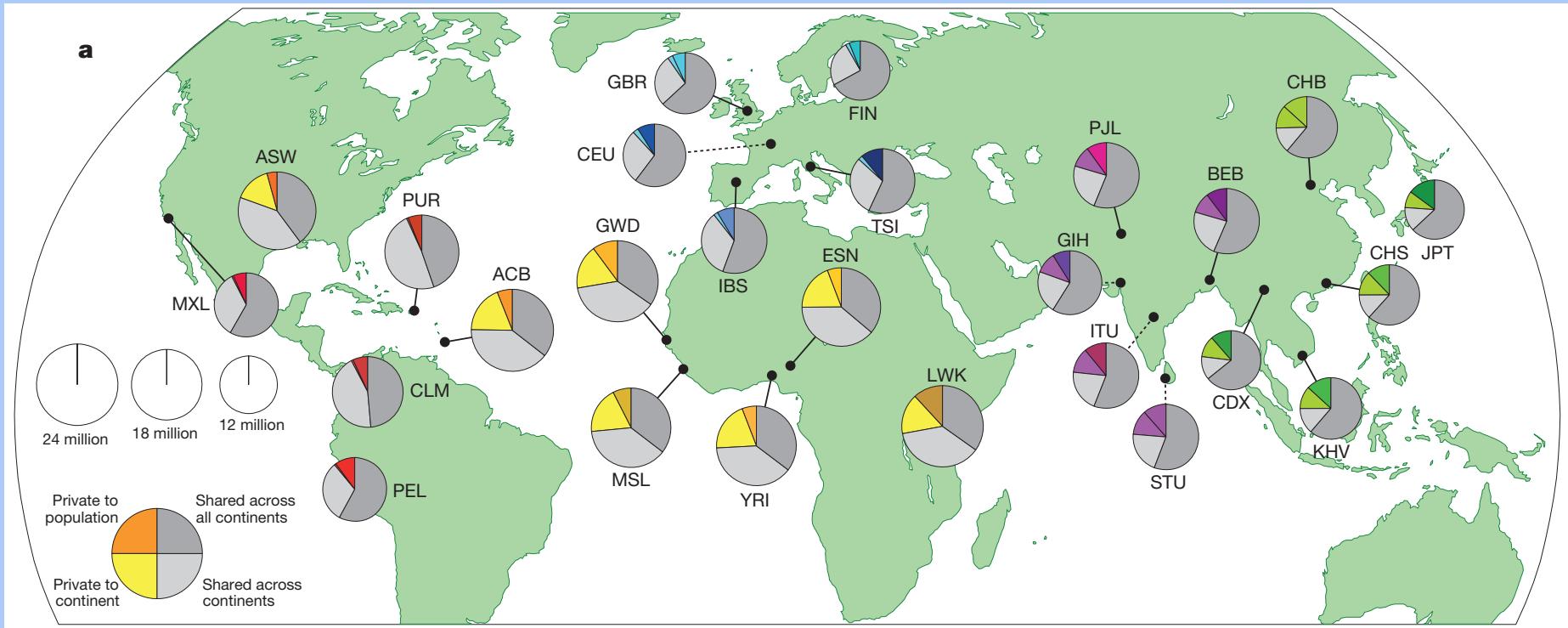


# The 1000 Genomes Project

- Phase 3 release (2015) contained data from 2,504 individuals representing 26 populations across the globe, and identified 85 million new SNPs



# Global genetic variation



- Most SNPs are shared across continents, and the majority of variation (~85%) is within rather than between populations

# The same yet different?

- Most variation is within-populations rather than between-populations
- Yet regional differences in allele frequencies lead to noticeable differences in phenotypes



# Statistical variation of an allele

- Variation of the counts  $x_i$  of an allele about the group mean  $\bar{x}_j$  and the population mean  $\bar{x}$

$$\sum_i (x_i - \bar{x})^2 = \sum_i (x_i - \bar{x}_{j(i)})^2 + \sum_i (\bar{x}_{j(i)} - \bar{x})^2$$

Total variation                    Within-population variation                    Between-population variation

# Pitfalls of not accounting for genetic ancestry

- Because of allele-frequency differences in global populations, **spurious associations** with disease risk can show up that may be entirely explained by genetic ancestry

# Example: lactase nonpersistence (lactose intolerance)

- The T allele of rs182549 is completely associated with the ability to digest lactose in Europeans

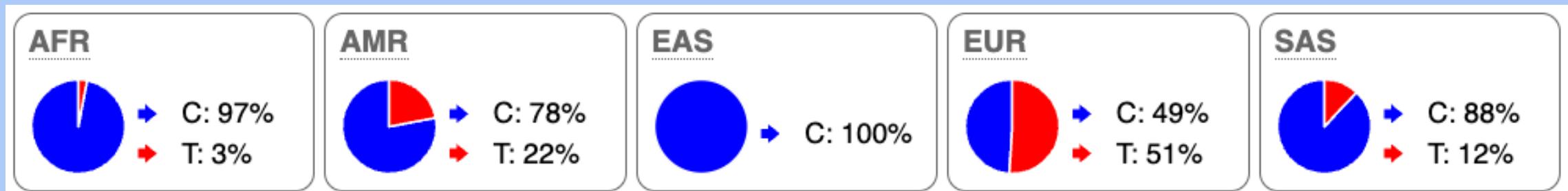
	CC	CT	TT
Non-persistence	59	0	0
Persistence	0	63	74

<https://pubmed.ncbi.nlm.nih.gov/11788828/>

# Example: lactase nonpersistence (lactose intolerance)

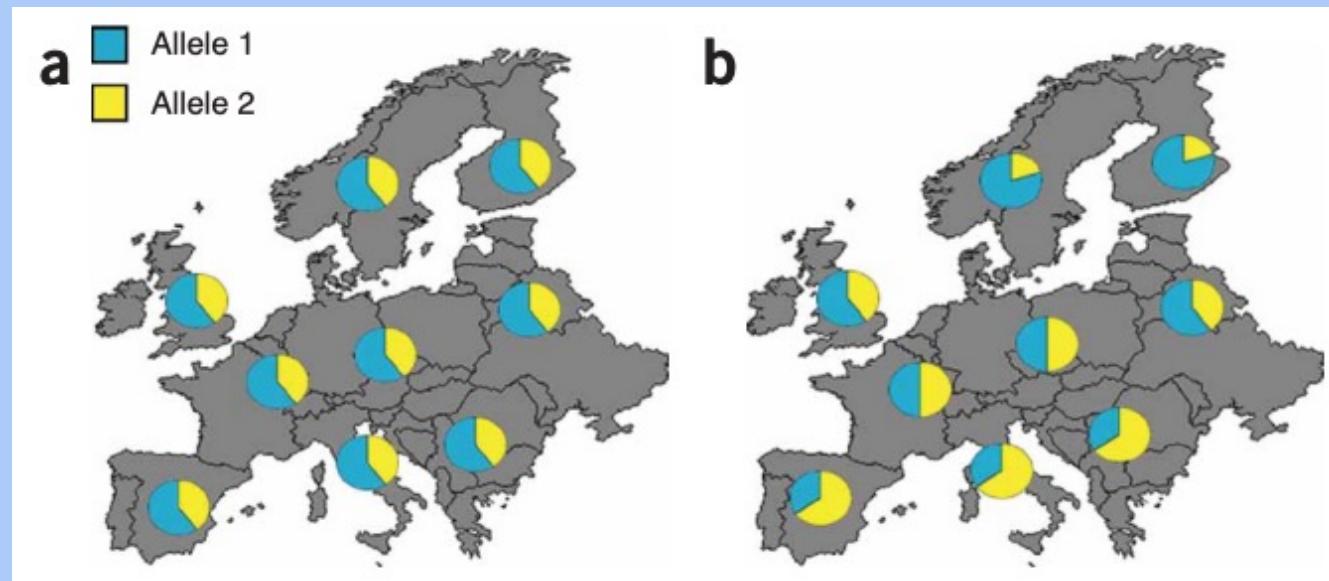
- Yet the polymorphism is almost absent in the African population, despite the presence of lactase persistence

<https://pubmed.ncbi.nlm.nih.gov/15106124/>



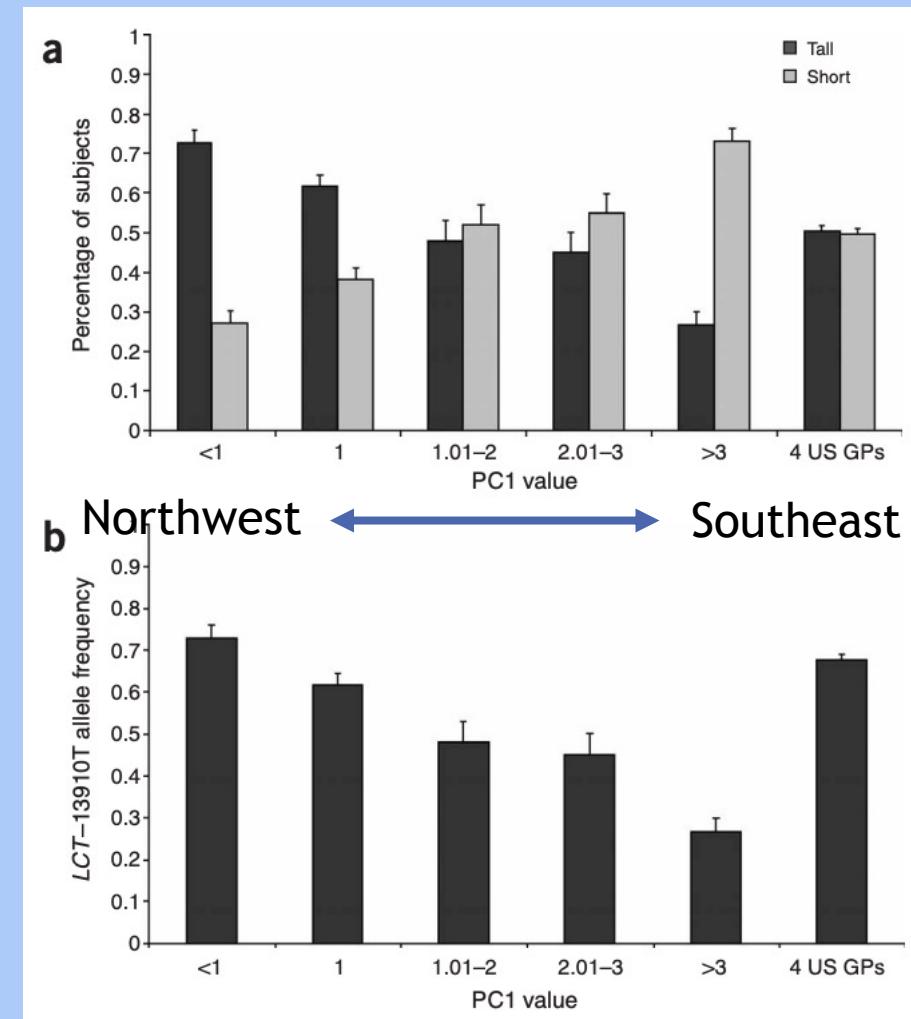
# Population stratification

- An allele may appear associated with a phenotype, when in fact it is associated with geographic origin (genetic ancestry)



# Spurious association

- An allele of the lactase-persistence SNP is spuriously associated with height, as its frequency is higher in individuals with Northern European ancestry vs. Southern



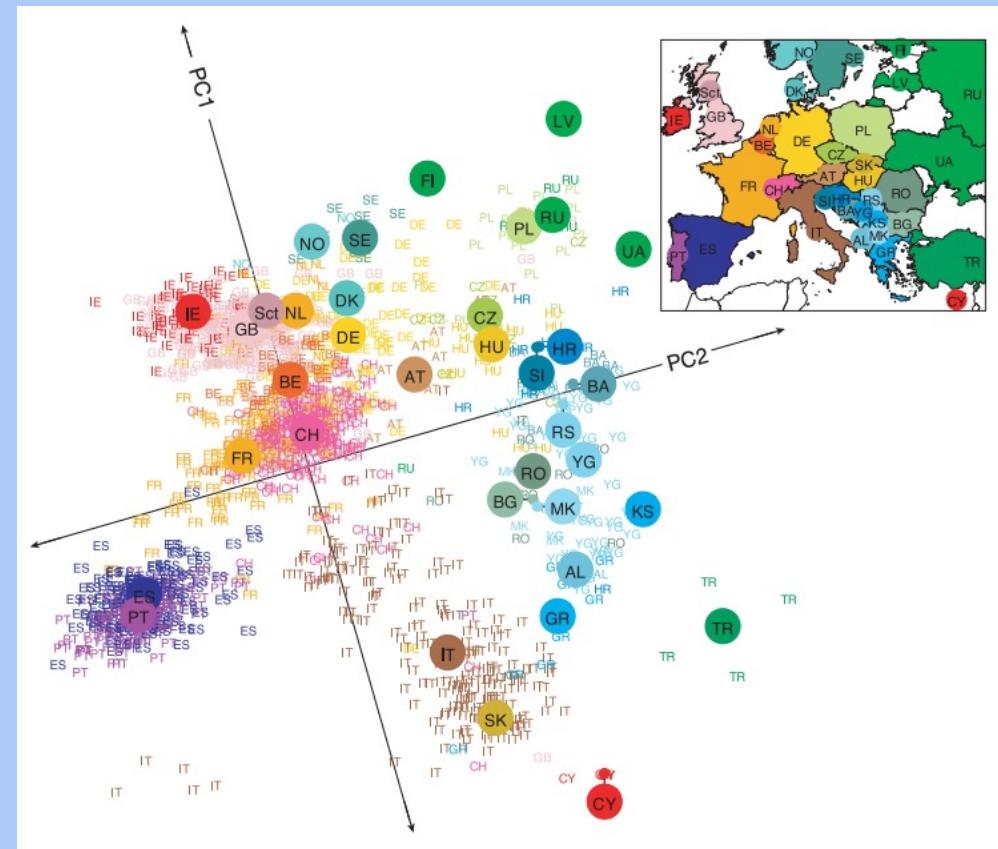
<https://pubmed.ncbi.nlm.nih.gov/16041375/>

# Principal components analysis

The concept of genetic ancestry

# Principal components analysis

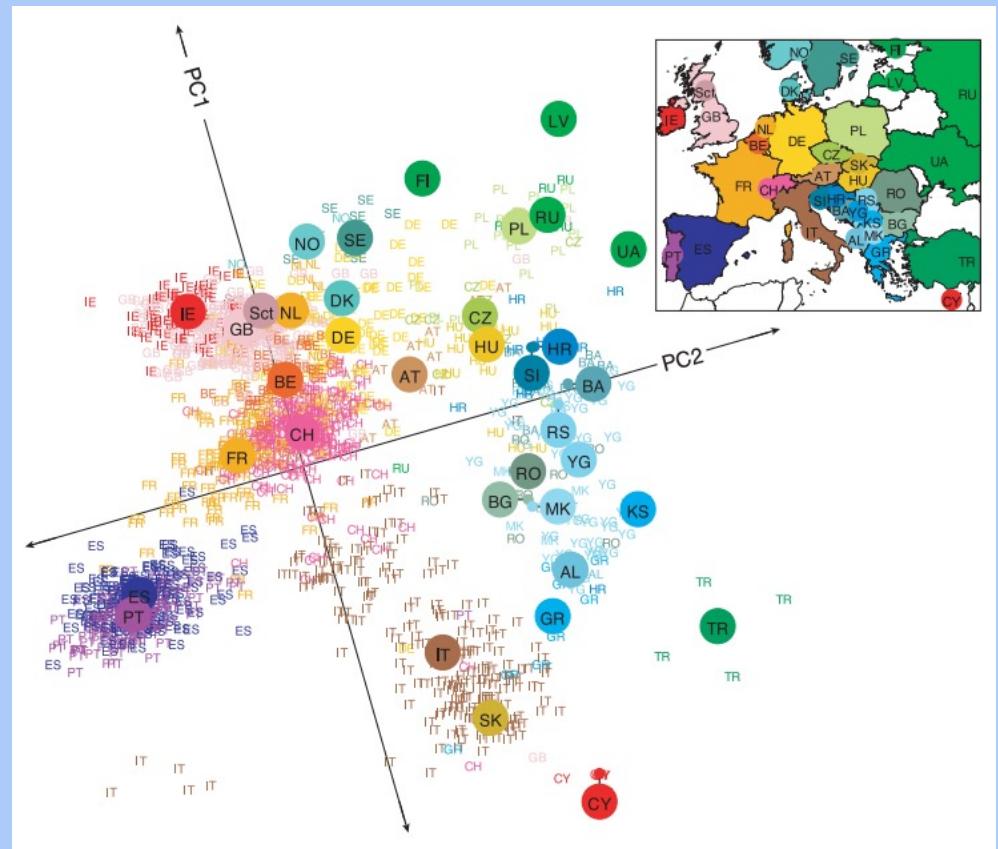
- Genotypes can distinguish population groups



<https://pubmed.ncbi.nlm.nih.gov/18758442/>

# Principal components analysis

- Looking at which variants segregate together can tell us about an individual's likely genetic ancestry



<https://pubmed.ncbi.nlm.nih.gov/18758442/>

# Genotype matrix

- $n$  individuals are genotyped at  $m$  SNPs

$$\mathbf{X} = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2m} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix}$$

m SNPs →

↓  
n subjects

# Genotype matrix

- The number of alternate alleles is 0, 1, or 2

$$\mathbf{X} = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2m} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix}$$

n subjects ↓      m SNPs →

# Genotype matrix

- “Standardize” each genotype by subtracting the mean allele (column) frequency and dividing by its standard error

$$\mathbf{X} = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2m} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix}$$

m SNPs →

↓ n subjects

# “Idealized” individuals

- An “idealized” subject of a particular genetic ancestry has genotypes  $v$  at  $m$  SNPs

$$\mathbf{X}\mathbf{V}^T = \begin{pmatrix} x_{11} & \cdots & x_{1m} \\ x_{21} & \cdots & x_{2n} \\ \vdots & & \vdots \\ x_{n1} & \cdots & x_{nm} \end{pmatrix} \begin{pmatrix} v_{11} & v_{12} & \cdots & v_{1n} \\ \vdots & \vdots & & \vdots \\ v_{m1} & v_{m2} & \cdots & v_{mn} \end{pmatrix}$$

# “Idealized” individuals

- The position  $u_{11}\lambda_{11}$  of individual 1 on PC1 is the “amount” of idealized person 1 in individual 1

$$\begin{pmatrix} u_{11} & \cdots & u_{1n} \\ u_{21} & \cdots & u_{2n} \\ \vdots & & \vdots \\ u_{n1} & \cdots & u_{nn} \end{pmatrix} \begin{pmatrix} \lambda_{11} & & & \\ & \lambda_{22} & & \\ & & \ddots & \\ & & & \lambda_{nn} \end{pmatrix} = \mathbf{U}\Sigma$$

# “Idealized” individuals

- The position  $u_{ij}\lambda_{jj}$  of individual i on PCj is the “amount” of idealized person j in individual i

$$\begin{pmatrix} u_{11} & \cdots & u_{1n} \\ u_{21} & \cdots & u_{2n} \\ \vdots & & \vdots \\ u_{n1} & \cdots & u_{nn} \end{pmatrix} \begin{pmatrix} \lambda_{11} & & & \\ & \lambda_{22} & & \\ & & \ddots & \\ & & & \lambda_{nn} \end{pmatrix} = \mathbf{U}\Sigma$$

# “Idealized” individuals

- The idea of PCA is to find the amount of each idealized individual in each actual individual using the decomposition of the  $n \times m$  genotype matrix  $\mathbf{X}$  into  $n \times n$ ,  $n \times n$ , and  $n \times m$  matrices  $\mathbf{U}$ ,  $\Sigma$ , and  $\mathbf{V}$

$$\mathbf{X}\mathbf{V}^T = \mathbf{U}\Sigma$$

# Genomic relationship matrix (GRM)

- The GRM is computed by comparing how similar any subject is to any other

$$\mathbf{X}\mathbf{X}^T = \begin{pmatrix} \mathbf{x}_1 \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_1 \cdot \mathbf{x}_n \\ \mathbf{x}_2 \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_2 \cdot \mathbf{x}_n \\ \vdots & & \vdots \\ \mathbf{x}_n \cdot \mathbf{x}_1 & \cdots & \mathbf{x}_n \cdot \mathbf{x}_n \end{pmatrix}$$

# Genomic relationship matrix (GRM)

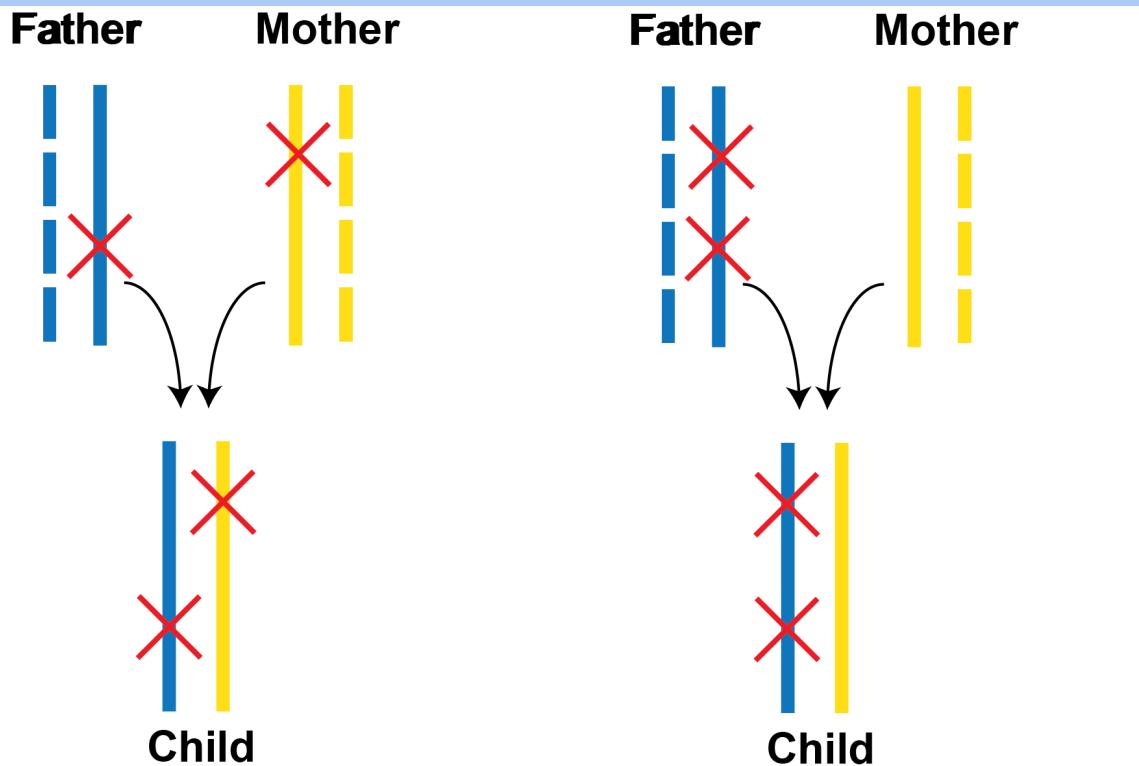
- The **eigenvectors** (columns of  $\mathbf{U}$ ) of the GRM contain the ancestry components

$$\mathbf{X}\mathbf{X}^T \mathbf{U} = \mathbf{U}\boldsymbol{\Sigma}^2$$

# Linkage disequilibrium

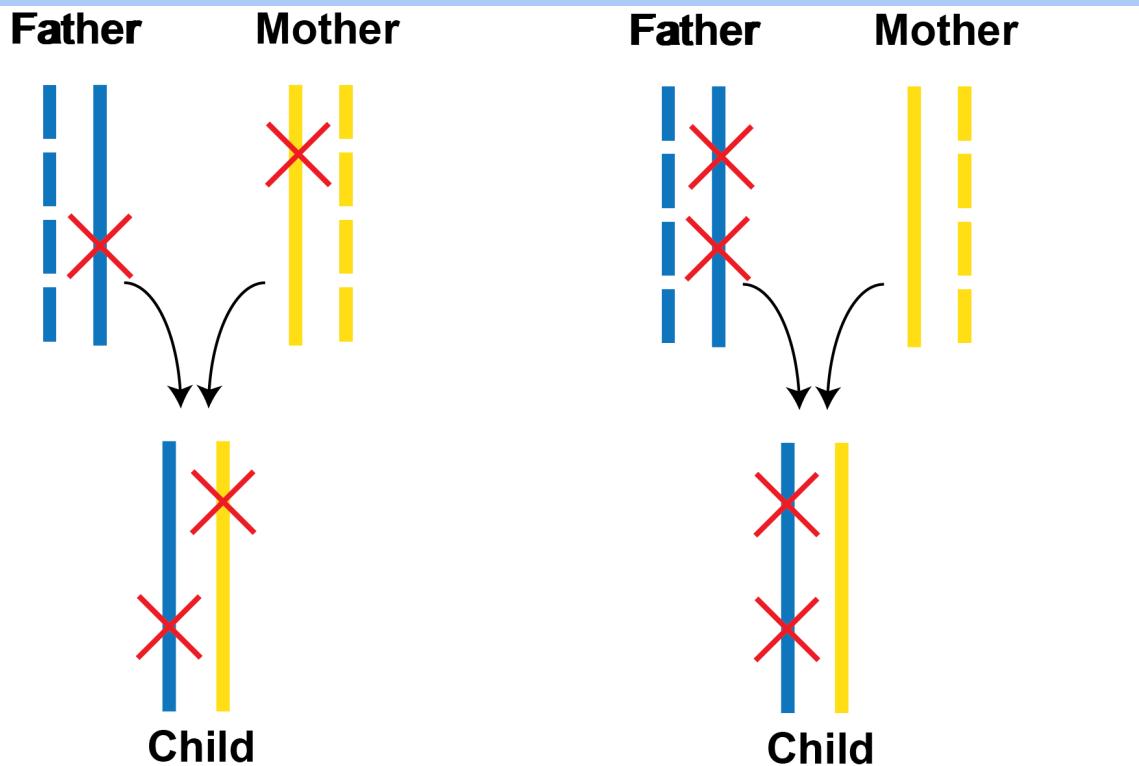
Determining a set of independent SNPs

# SNPs can occur on either of two chromosomes



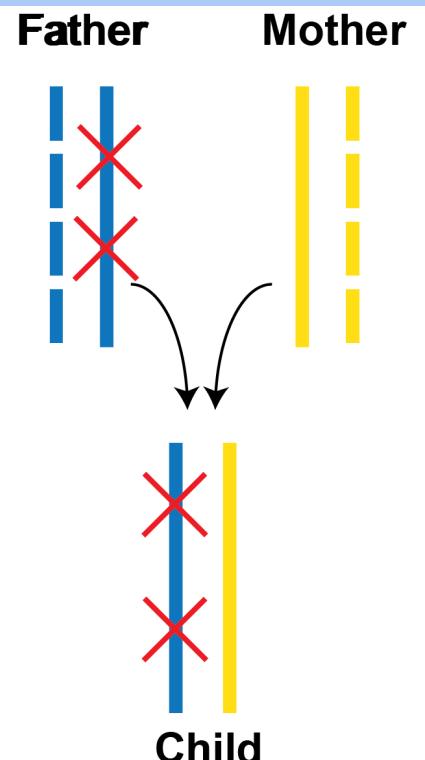
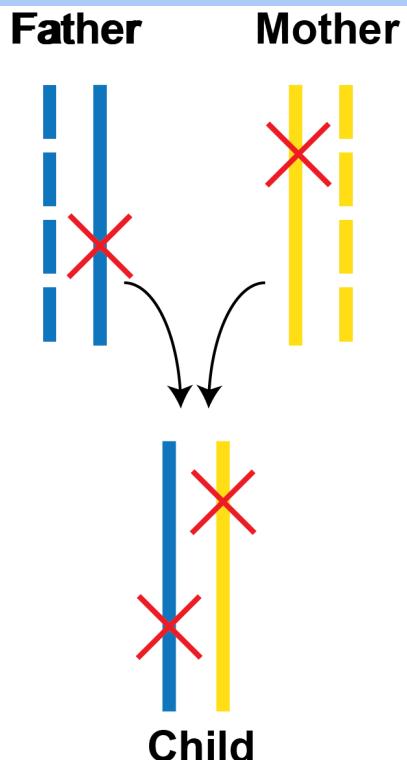
- Genotype data do not tell us which chromosomes carry the polymorphism

# SNPs can occur on either of two chromosomes



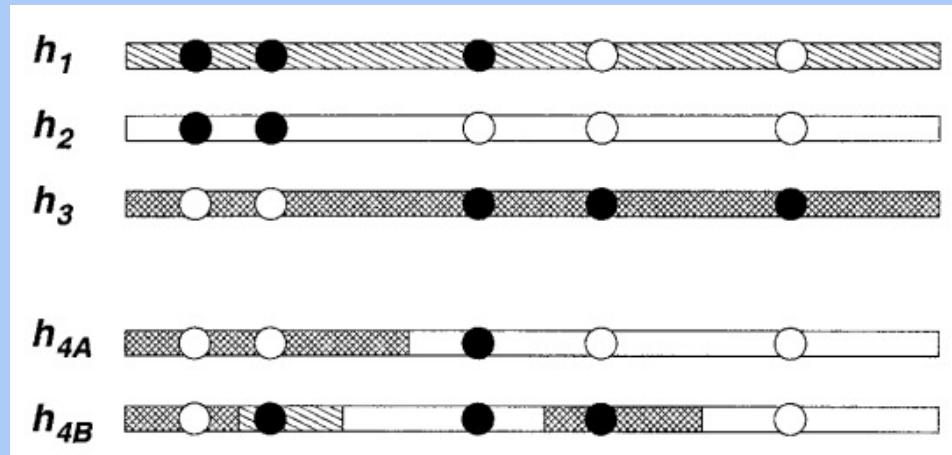
- When at least one parent is homozygous at each SNP, **haplotype phase** can be unambiguously assigned

# SNPs can occur on either of two chromosomes



- and we can distinguish AB/ab from Ab/aB

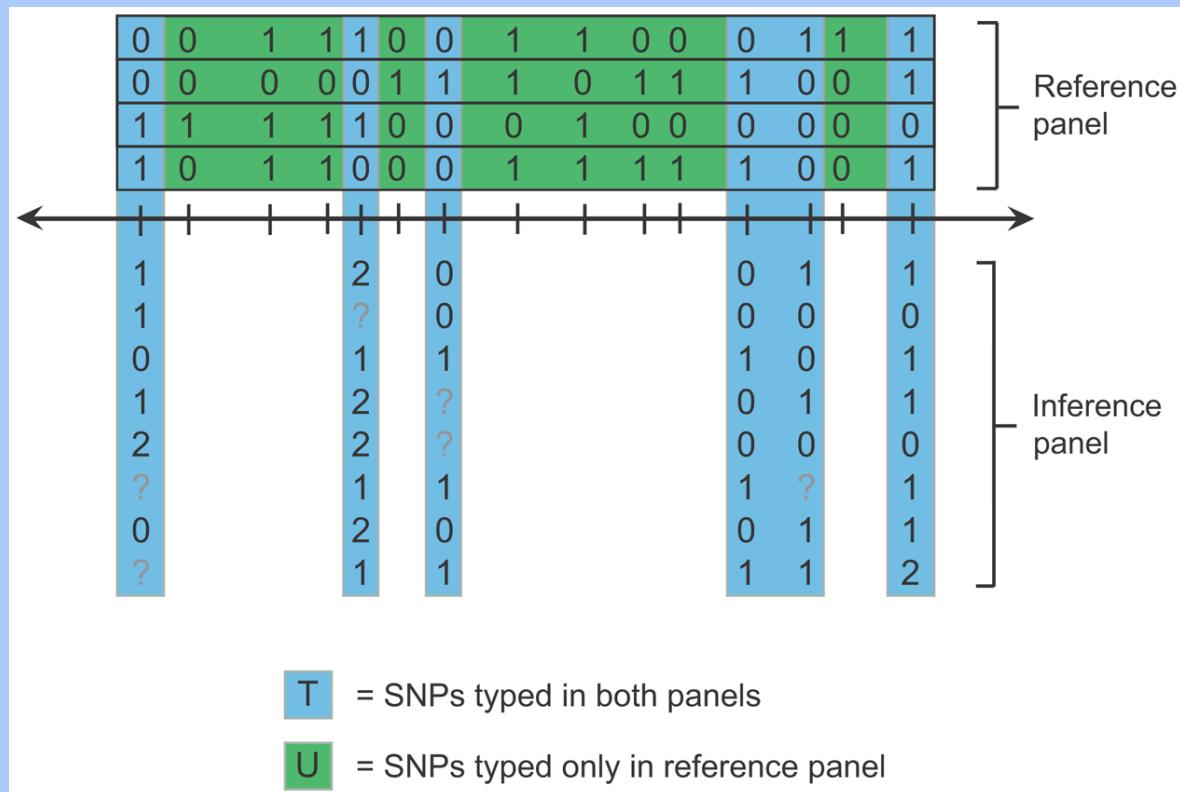
# Statistical phasing and imputation



<https://pubmed.ncbi.nlm.nih.gov/14704198/>

- Genotyped individuals can be computationally phased by modelling each chromosome as an imperfect mosaic of chromosomes from a reference panel

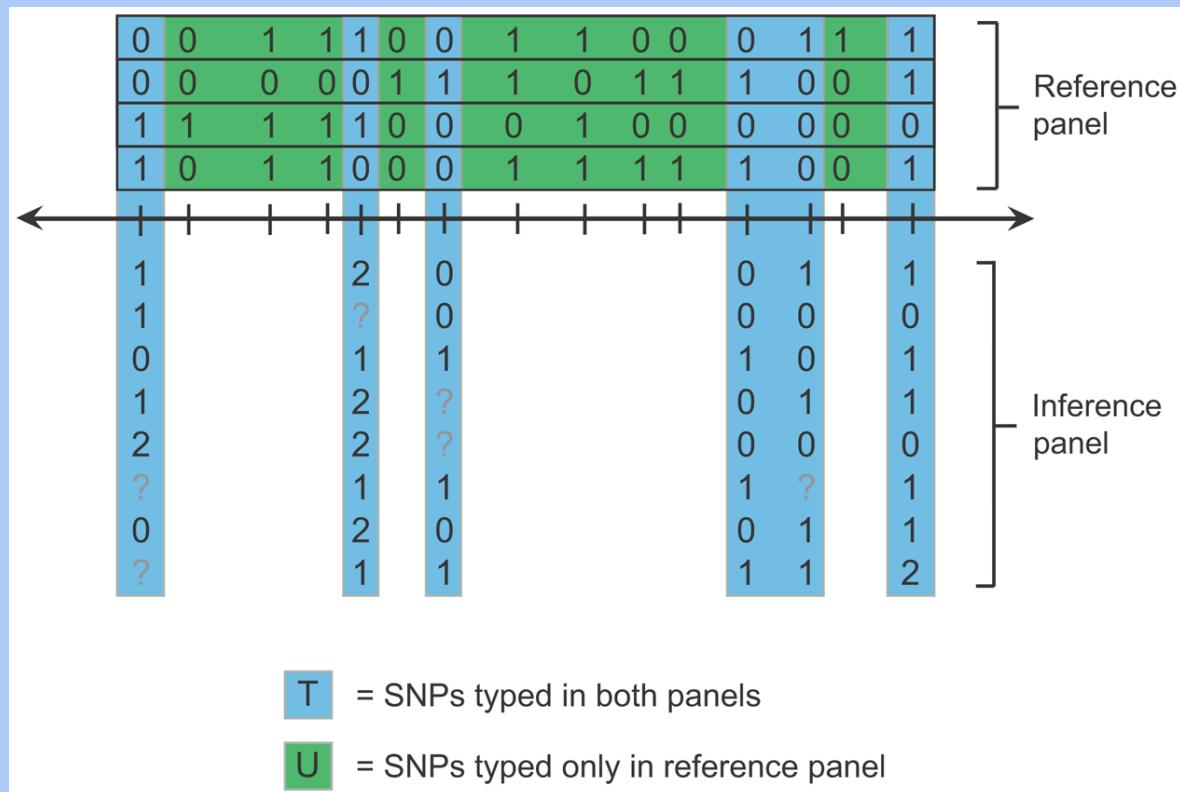
# Statistical phasing and imputation



- Variants that have not been typed can be **imputed** into the inference sample

<https://pubmed.ncbi.nlm.nih.gov/19543373/>

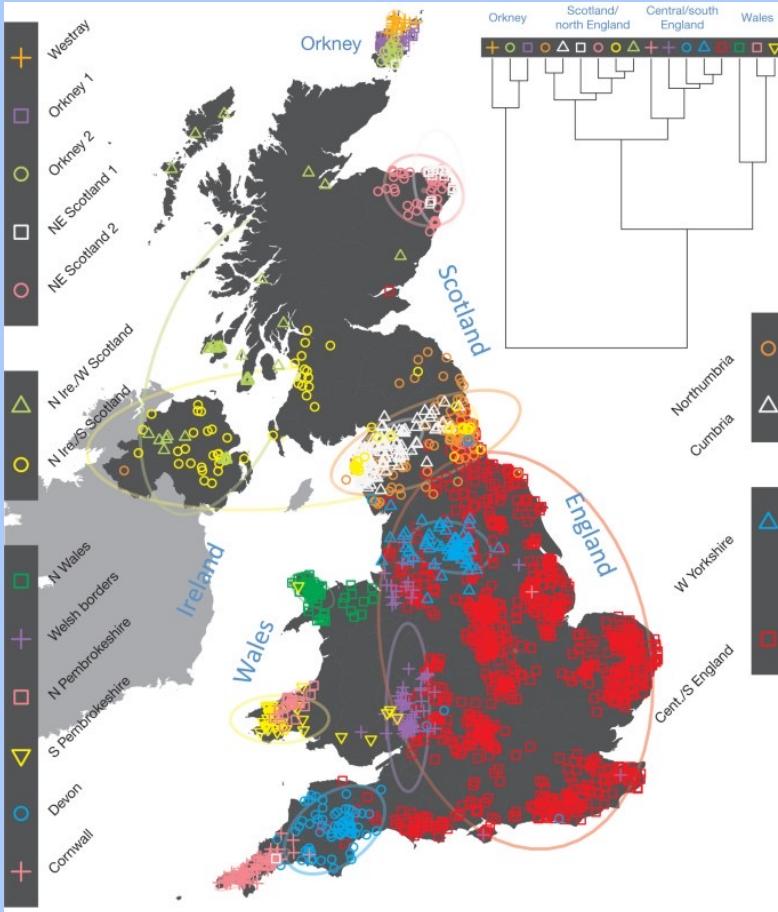
# Statistical phasing and imputation



- Imputation accuracy depends on the inference and reference samples being of similar genetic ancestry

<https://pubmed.ncbi.nlm.nih.gov/19543373/>

# Different haplotypes distinguish different populations



- Individuals can be grouped into populations with which they have the most haplotype-sharing

<https://pubmed.ncbi.nlm.nih.gov/25788095/>

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# Linkage disequilibrium

- Linkage disequilibrium is the population tendency of alleles to be inherited on a single chromosome

# Linkage disequilibrium

- LD is measured as the correlation coefficient between the alleles of different SNPs

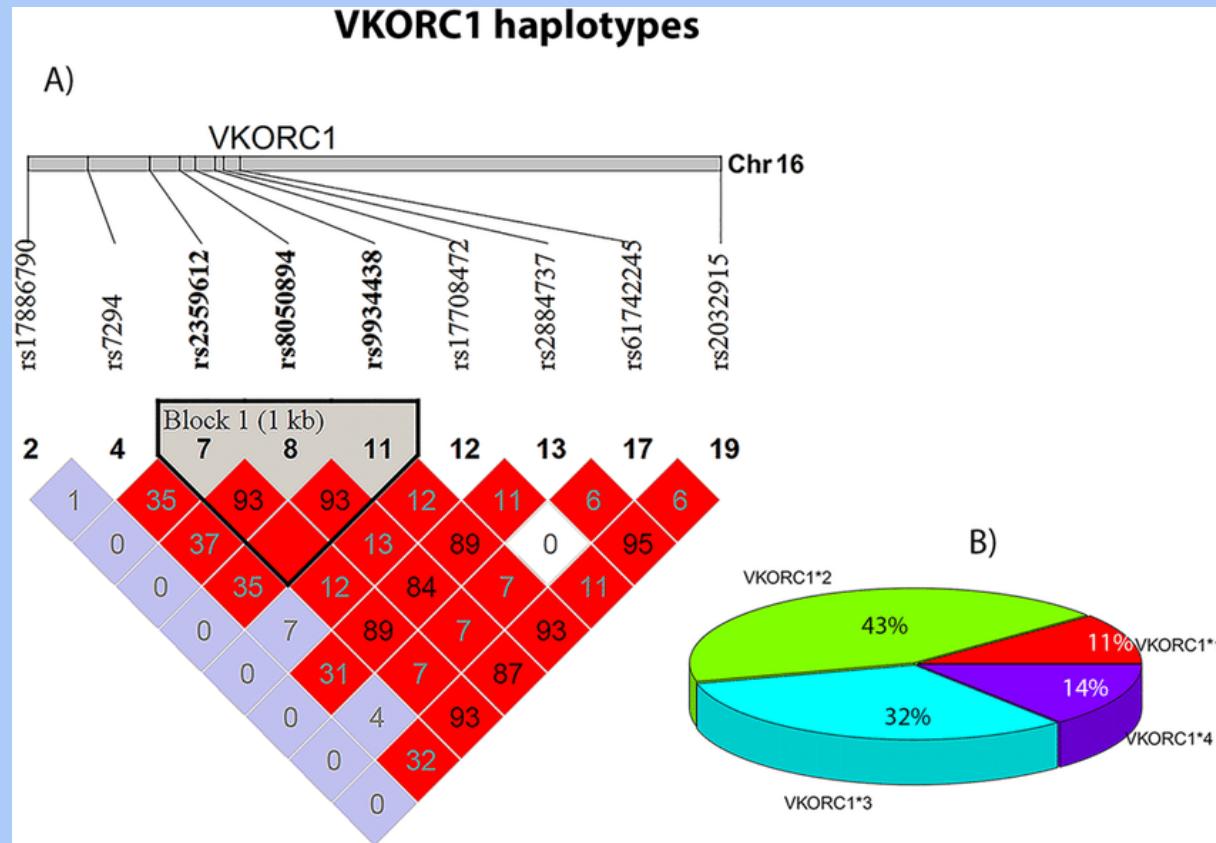
$$r_{A,B} = \frac{p_{A,B} - p_A p_B}{\sqrt{p_A (1 - p_A) p_B (1 - p_B)}}$$

# Linkage disequilibrium

- $p_A$  = fraction of chromosomes with A
- $p_{AB}$  = fraction of chromosomes with A and B

$$r_{A,B} = \frac{p_{A,B} - p_A p_B}{\sqrt{p_A (1 - p_A) p_B (1 - p_B)}}$$

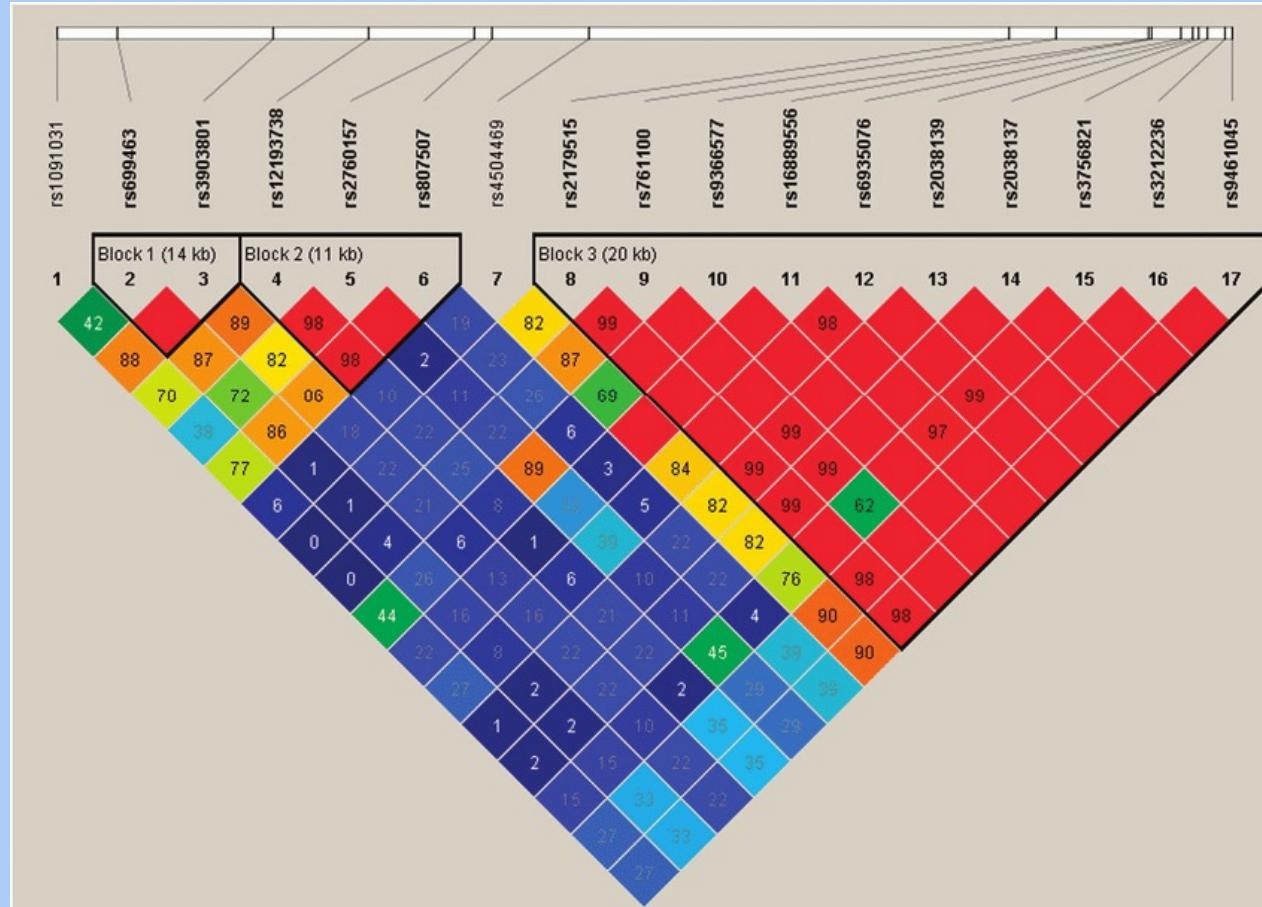
# LD blocks and haplotype structure



<https://pubmed.ncbi.nlm.nih.gov/32221414/>

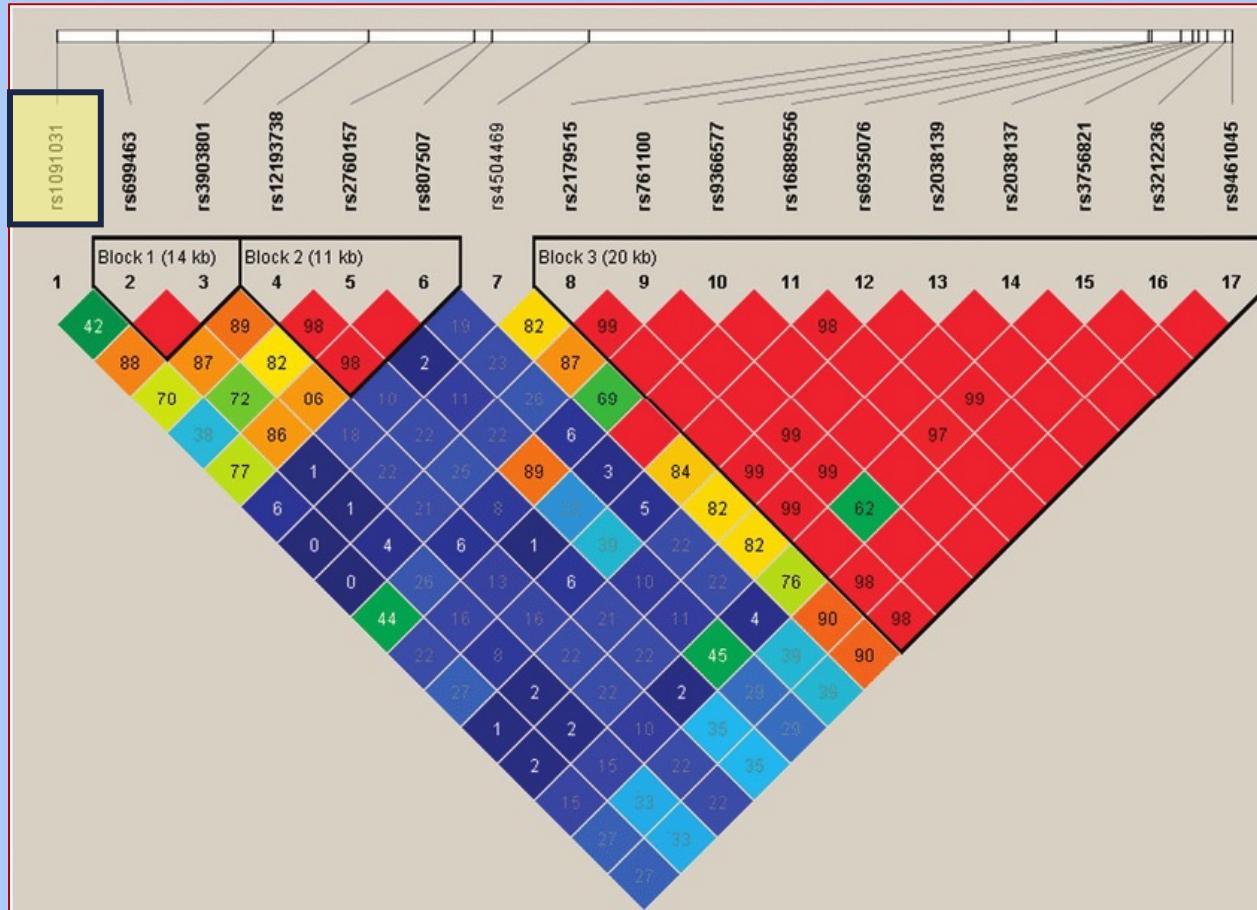
- Plots of pairwise  $r^2$  values show which SNPs are inherited together in the population as common haplotypes

# An algorithm for computing an independent subset of alleles



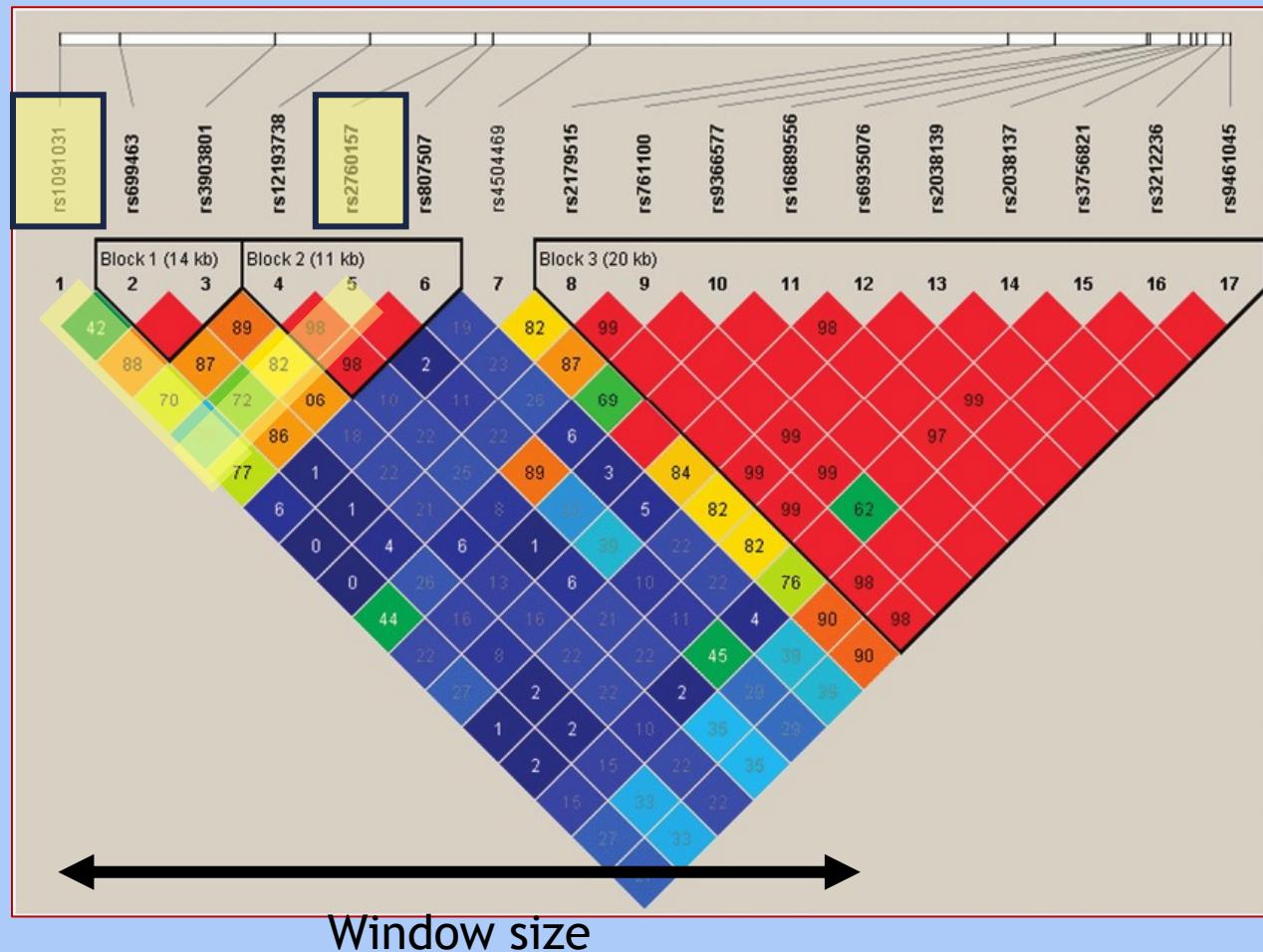
- From the SNPRelate package  
<https://rdrr.io/bioc/SNPRelate/man/snpgdsLDpruning.html>

# An algorithm for computing an independent subset of alleles



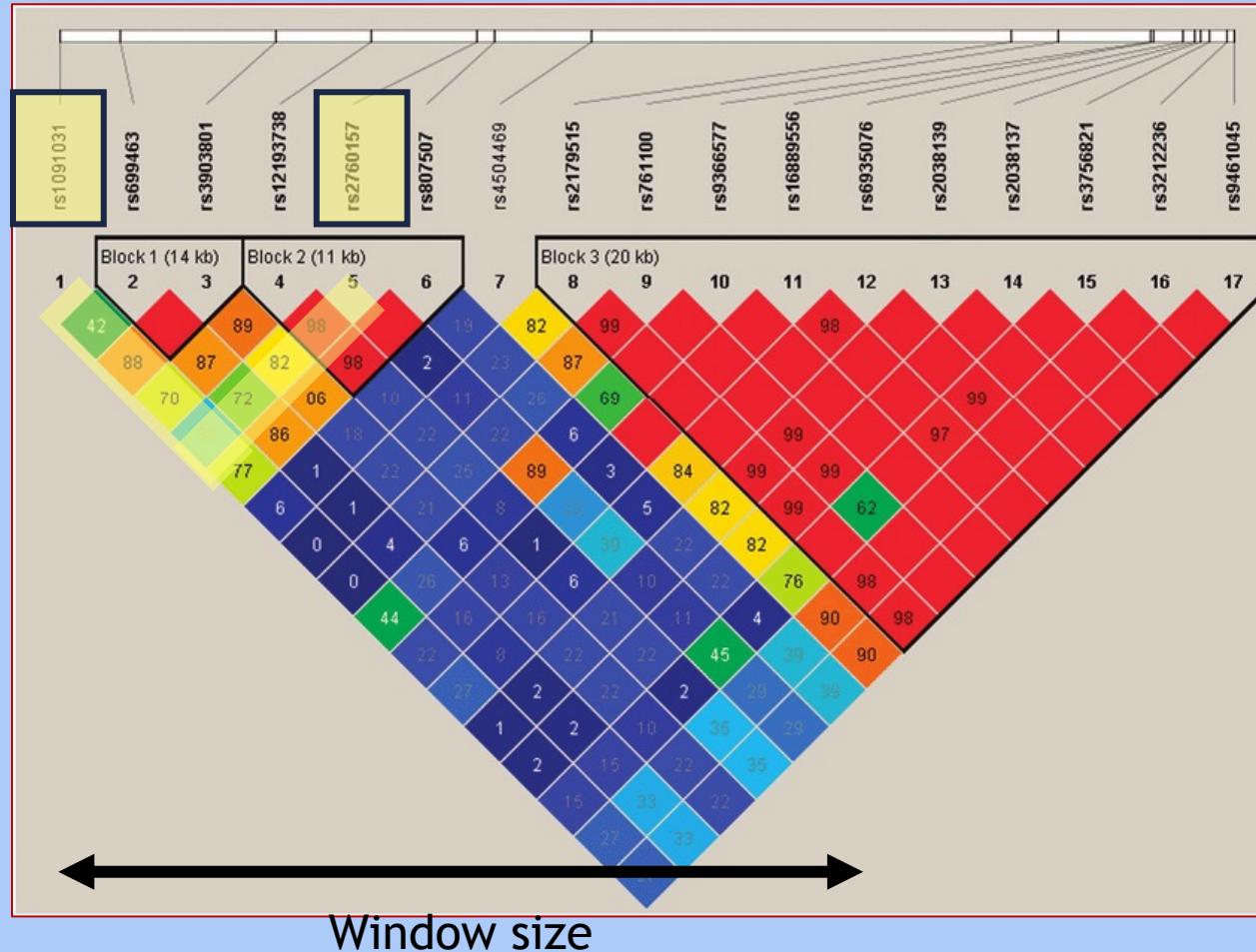
- Pick a random SNP

# An algorithm for computing an independent subset of alleles



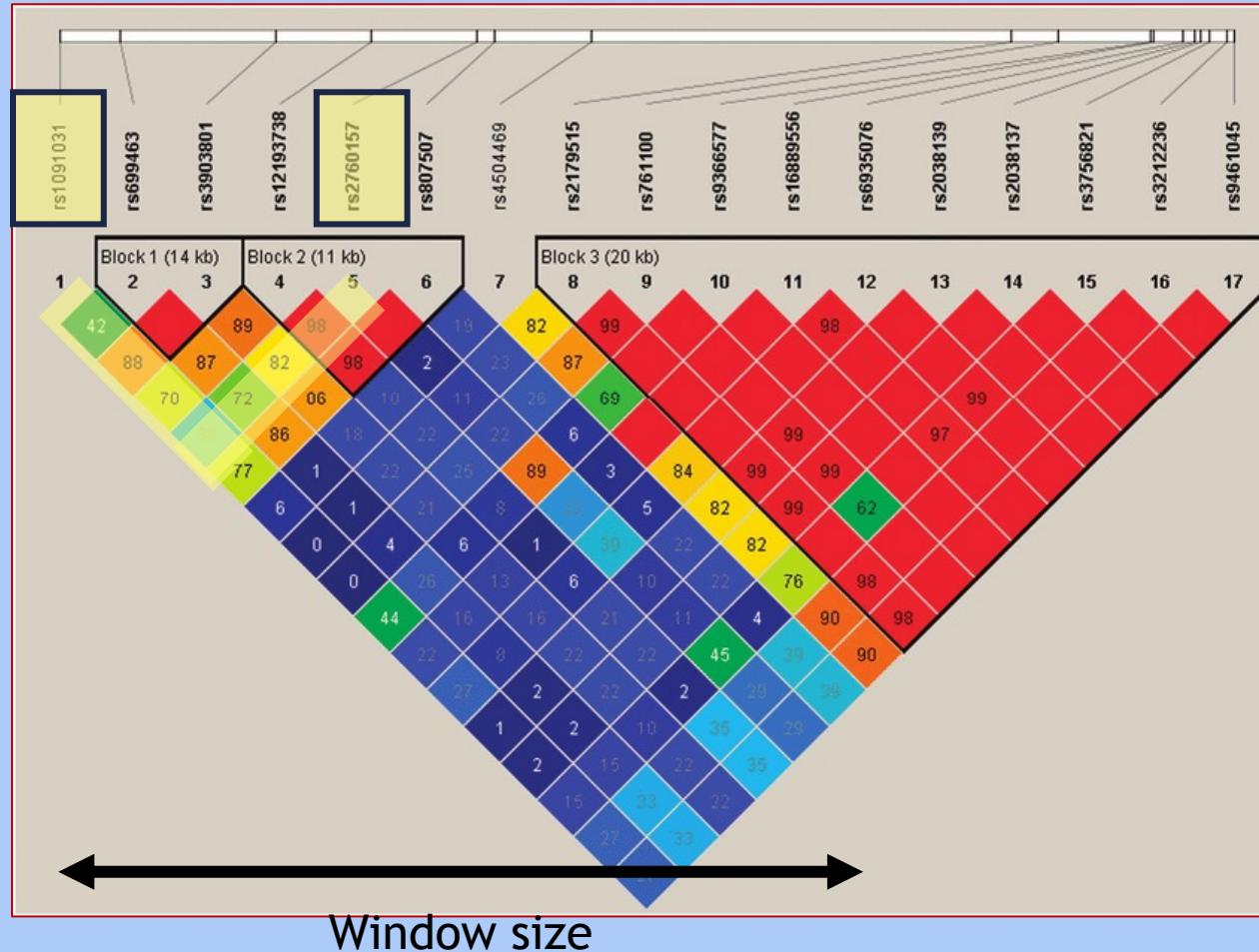
- Compute the LD with every other SNP within a sliding window of predetermined size

# An algorithm for computing an independent subset of alleles



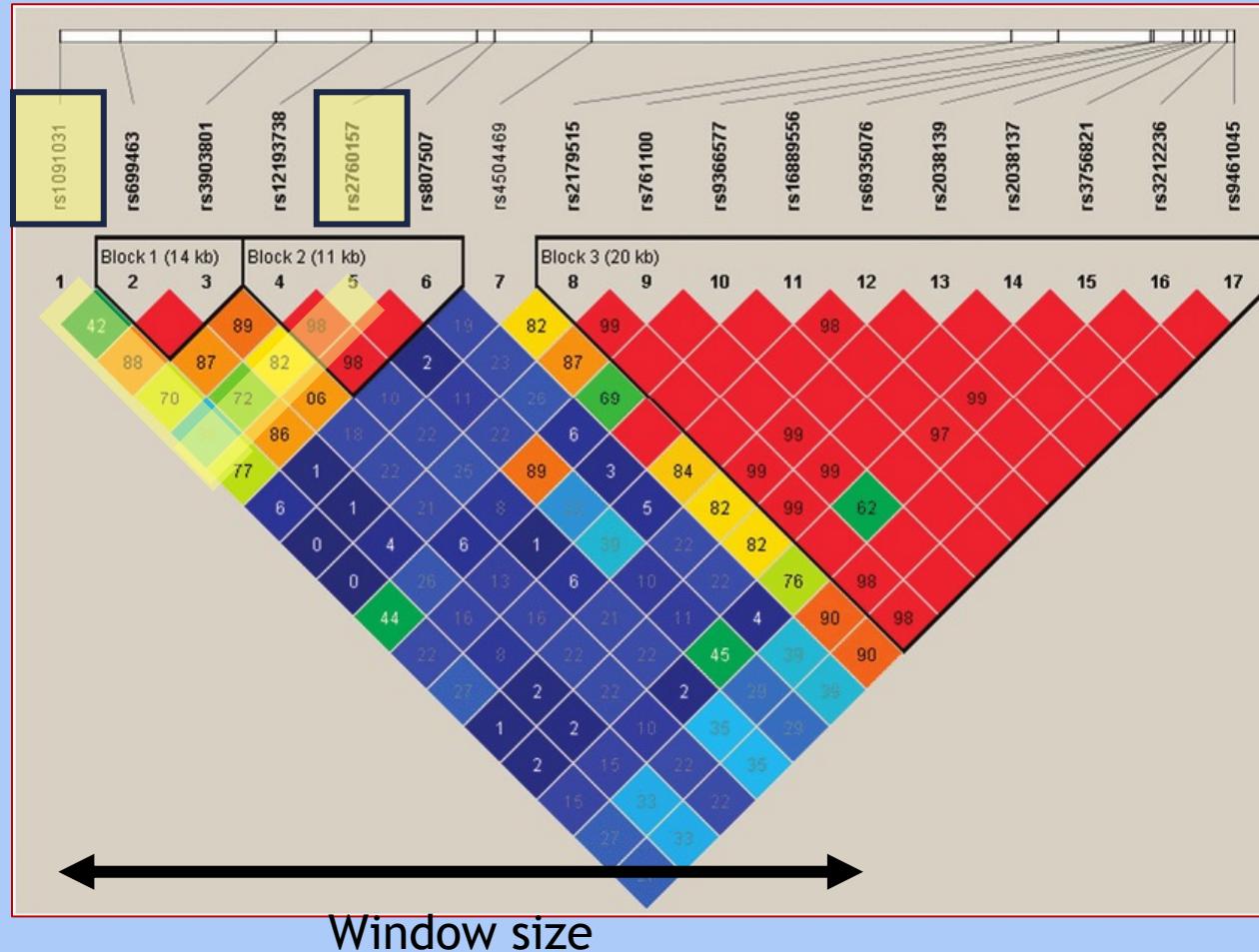
- If  $LD > \text{threshold}$ , remove the SNP

# An algorithm for computing an independent subset of alleles



- Else it becomes a new independent SNP

# An algorithm for computing an independent subset of alleles

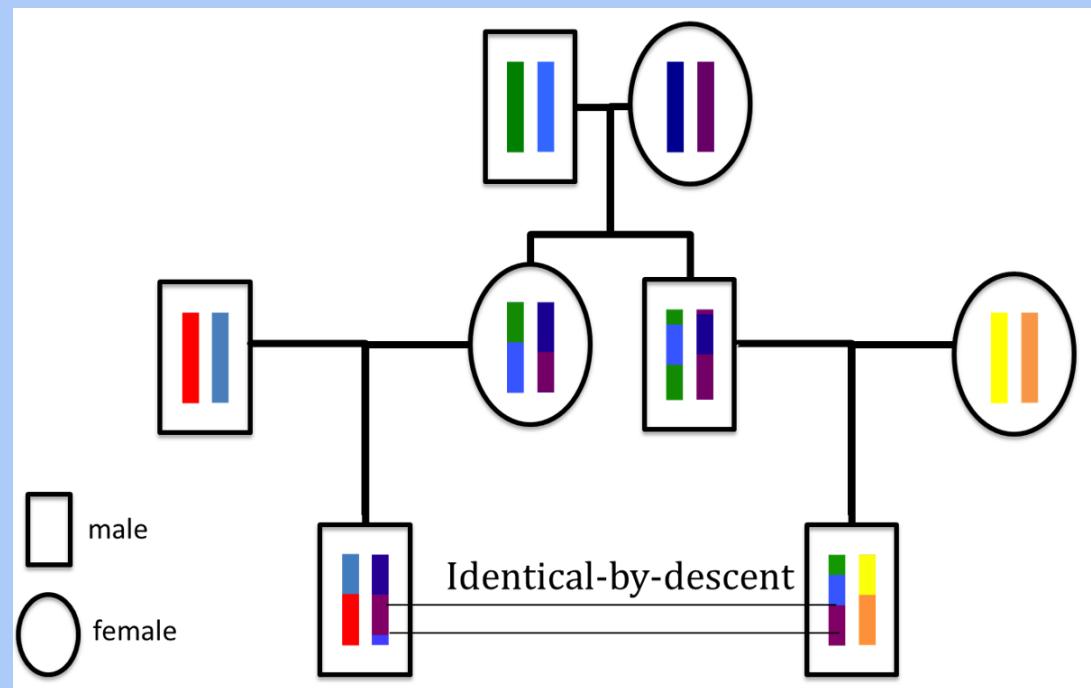


- The algorithm is random, and should be initiated from fixed seed

# Kinship analysis

## The concept of genetic relatedness

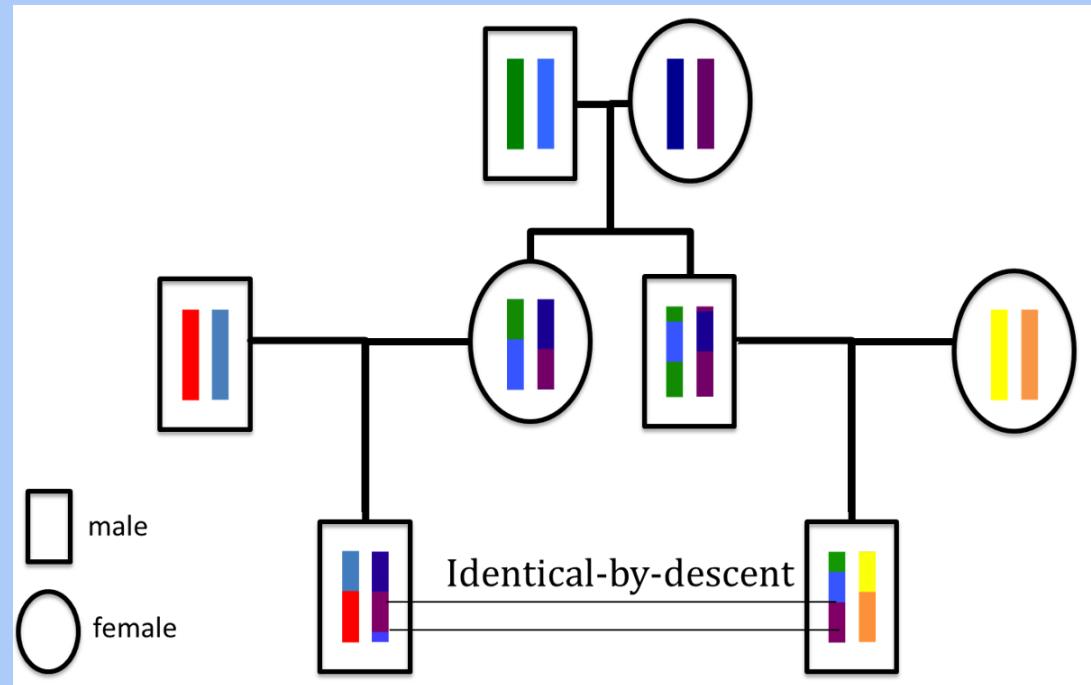
# Relatives share haplotypes IBD



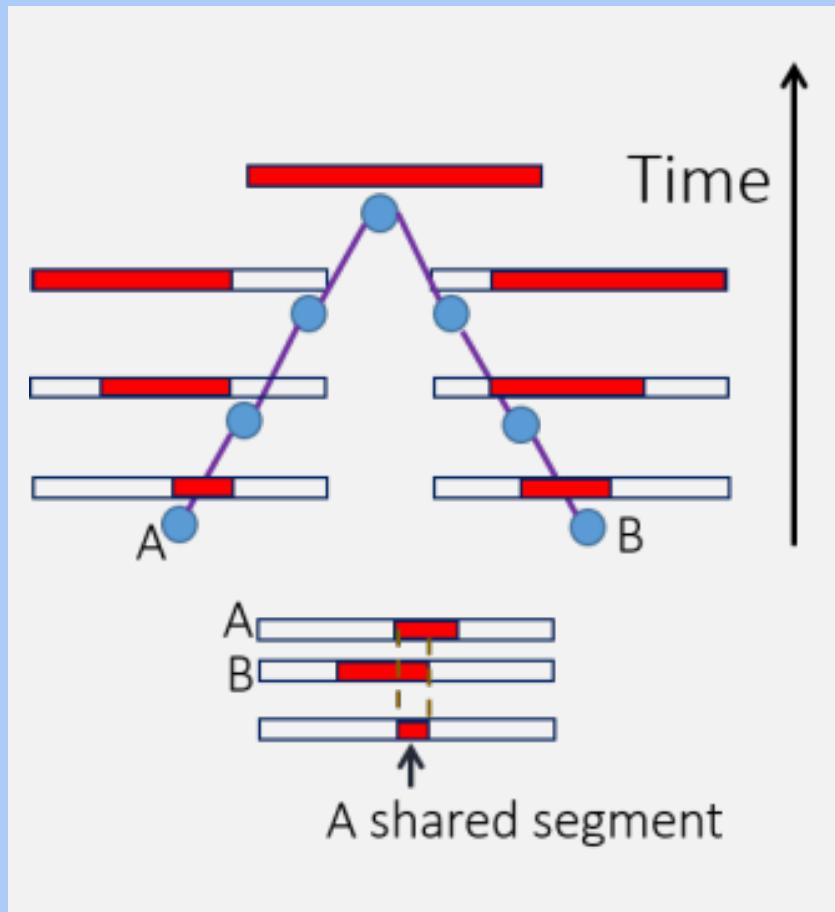
- Segments of DNA inherited from a common ancestor are said to be **identical by descent (IBD)**

# Relatives share haplotypes IBD

- DNA that just happens to be the same is **identical by state (IBS)**



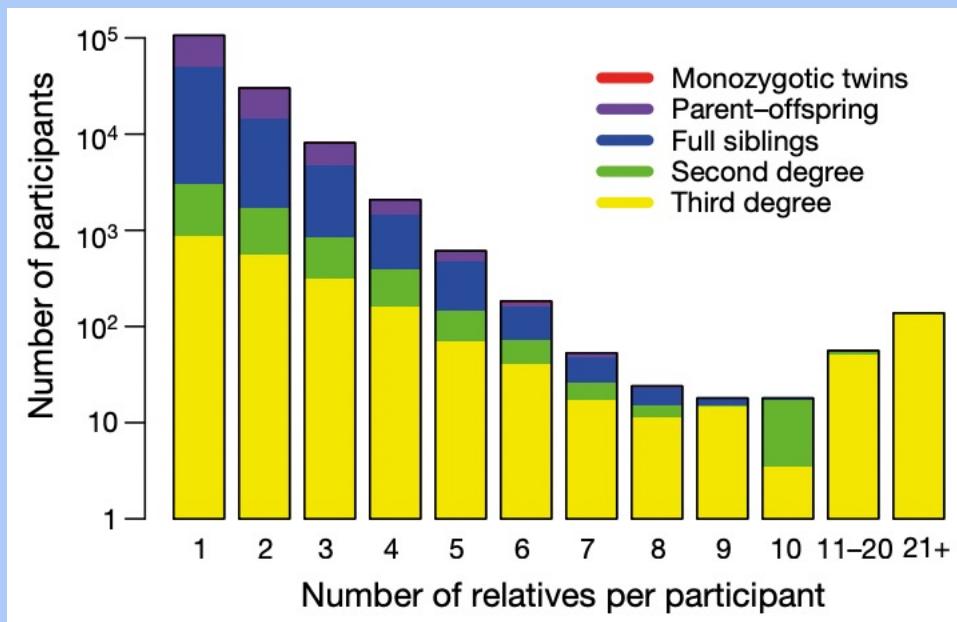
# Haplotype sharing decays over time



- The longer the IBD segment, the more closely related are the two individuals

# Kinship in genetic association studies

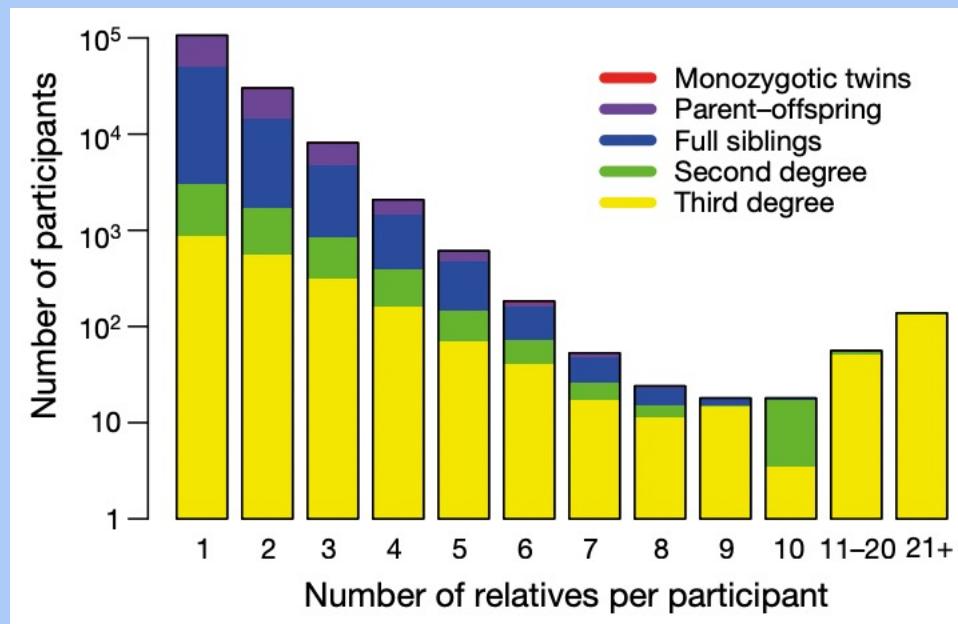
- Large genomic datasets, such as the UK Biobank, contain related individuals



<https://pubmed.ncbi.nlm.nih.gov/30305743/>

# Kinship in genetic association studies

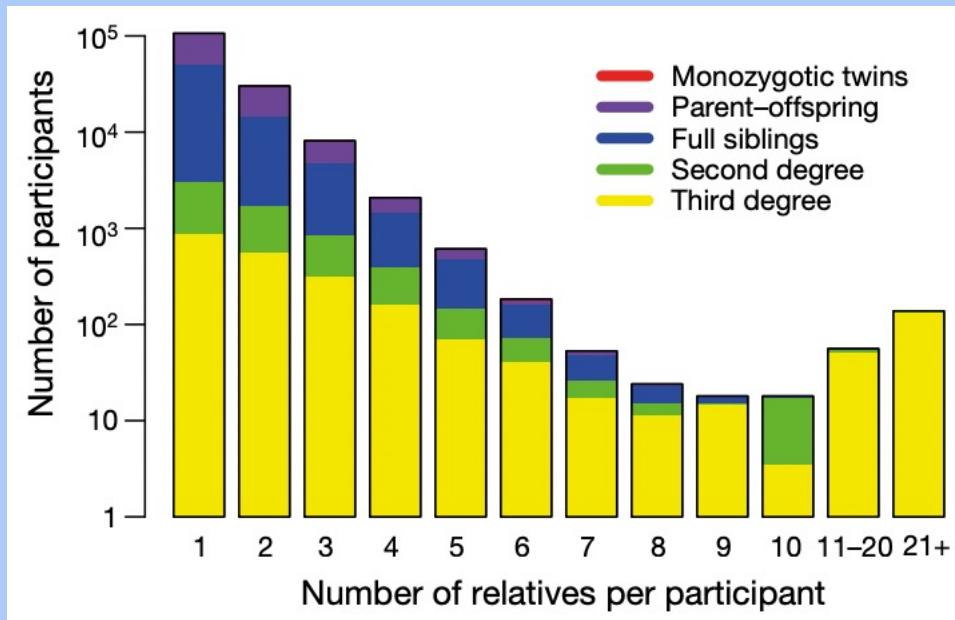
- Sometimes there is even “cryptic” relatedness



<https://pubmed.ncbi.nlm.nih.gov/30305743/>

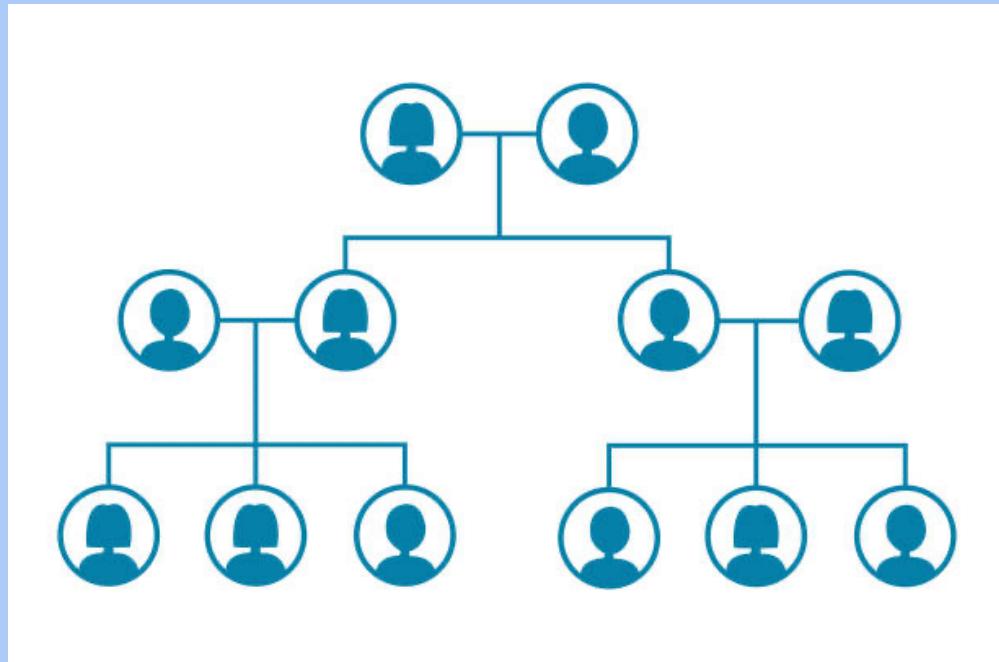
# Kinship in genetic association studies

- Because of IBD sharing, not all the observations are independent, and genotype-phenotype associations may be confounded



<https://pubmed.ncbi.nlm.nih.gov/30305743/>

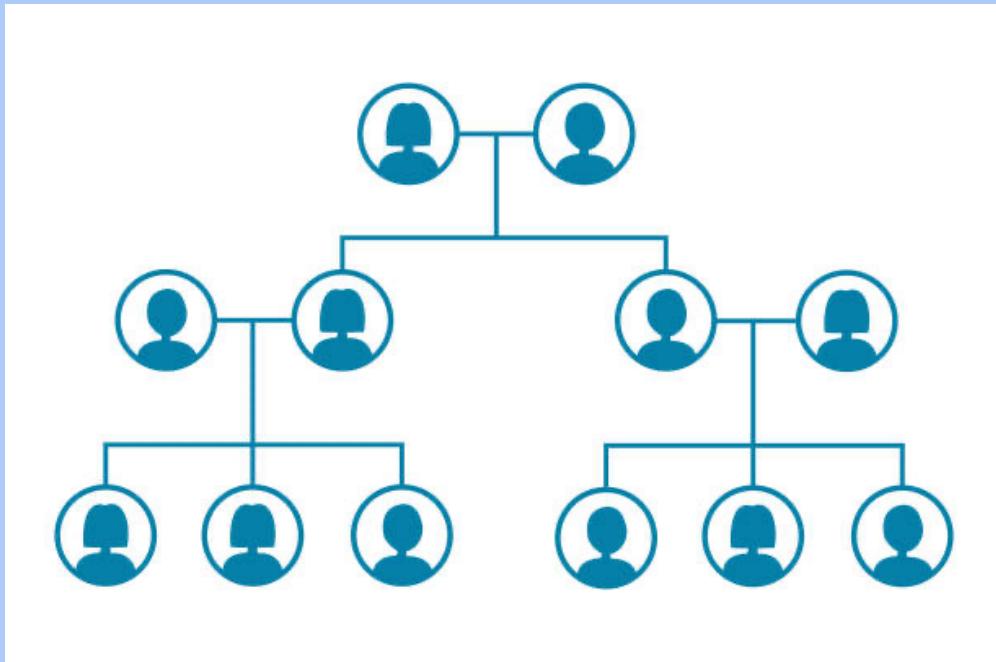
# Degree of relatedness



- R is the **effective number of meioses** separating two individuals through their two parents 1 and 2

$$\frac{1}{2^R} = \frac{1}{2^{R_1}} + \frac{1}{2^{R_2}}$$

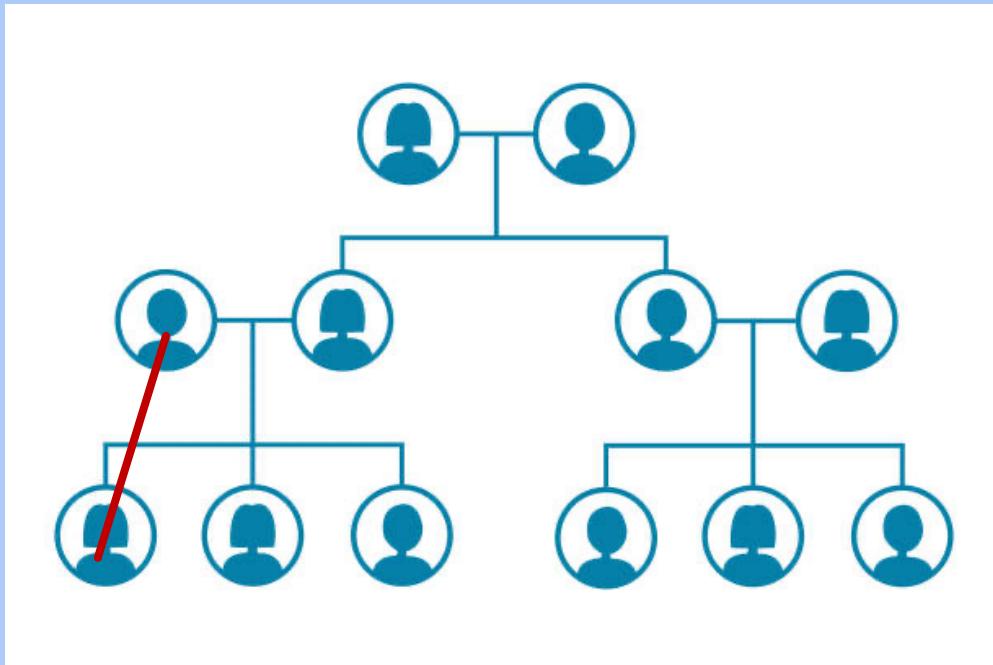
# Degree of relatedness



- $R \rightarrow \infty$  for unrelated individuals

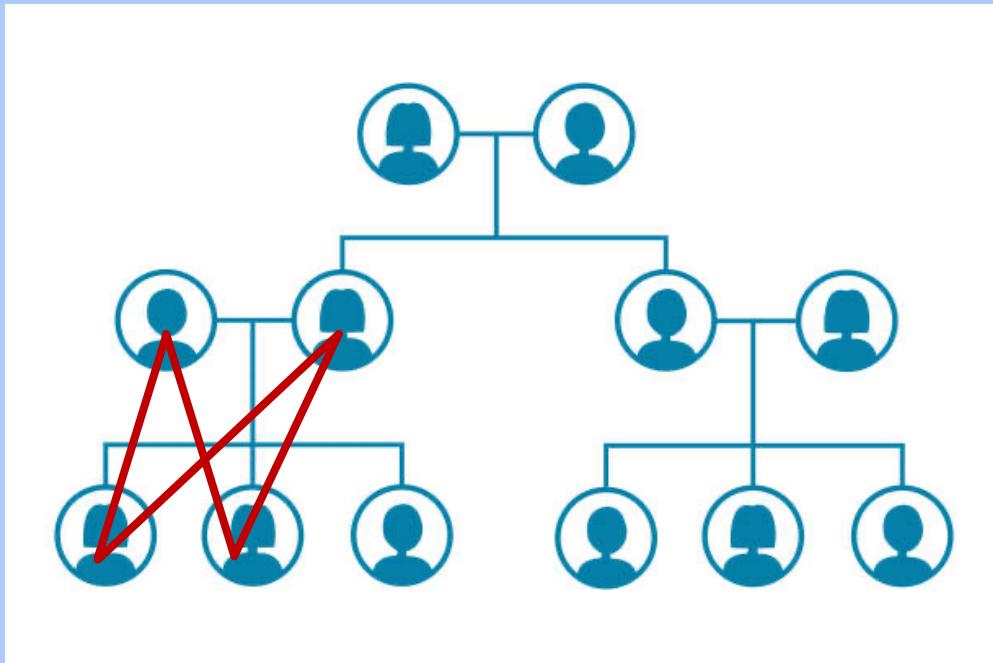
$$\frac{1}{2^R} = \frac{1}{2^{R_1}} + \frac{1}{2^{R_2}}$$

# Degree of relatedness



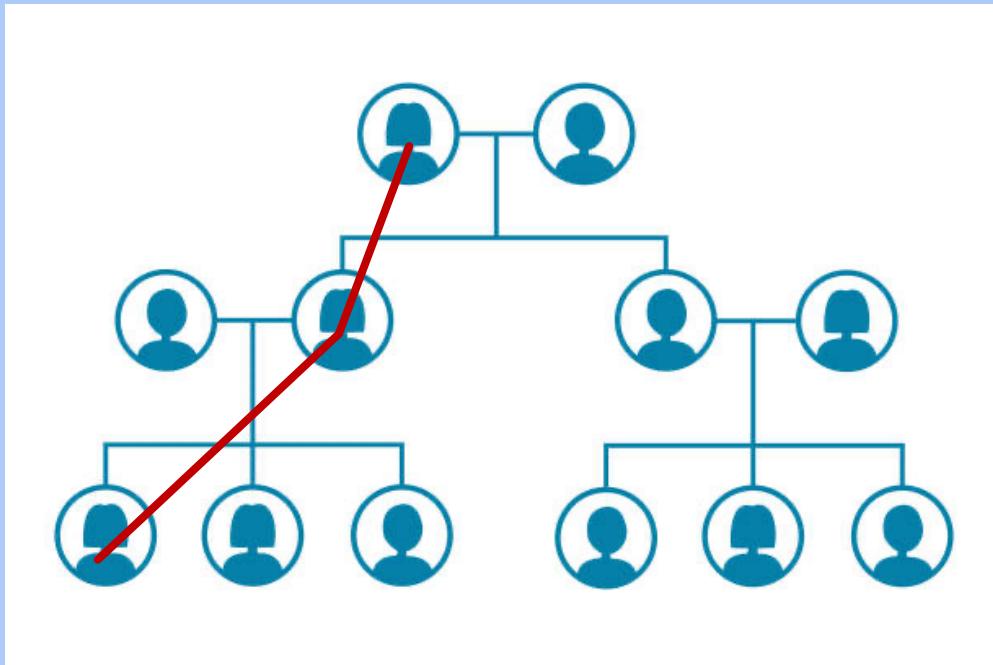
- Parent-child:
  - $R = 1$  meiosis

# Degree of relatedness



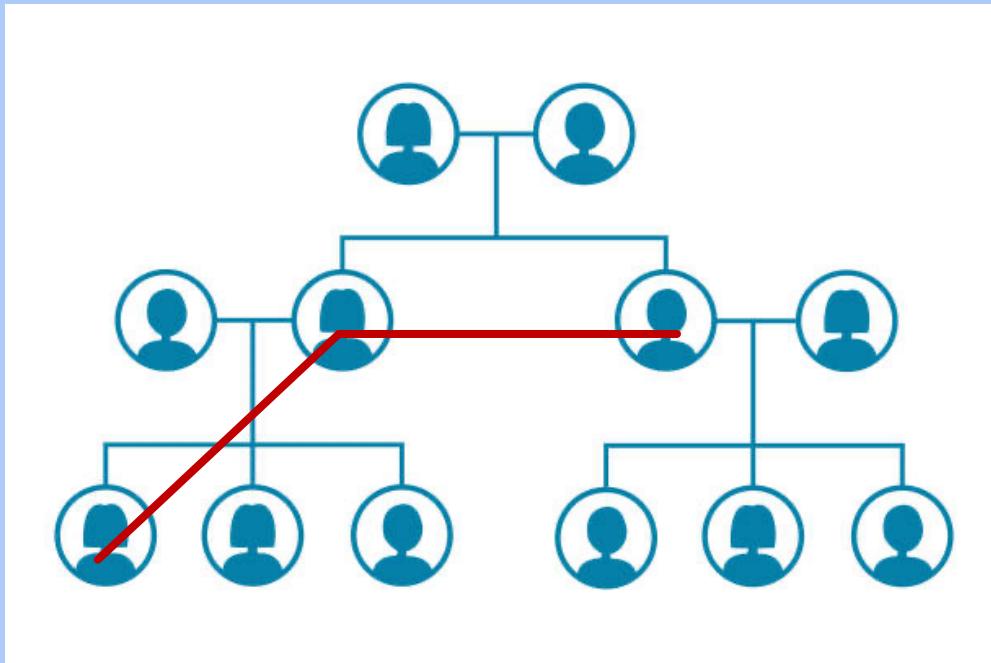
- Siblings:  $R = 1$  “effective” meiosis:
  - $1 / 2^1 = 1 / 2^2 + 1 / 2^2$

# Degree of relatedness



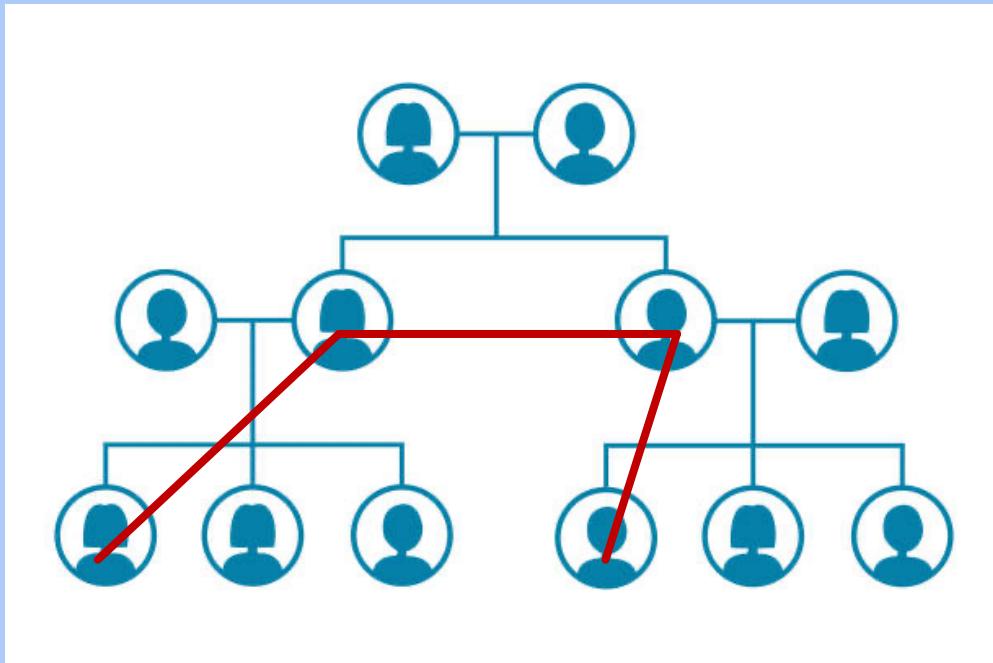
- Grandparent-grandchild:
  - $R = 2$  meioses

# Degree of relatedness



- Avuncular:
  - $R = 2$  meioses

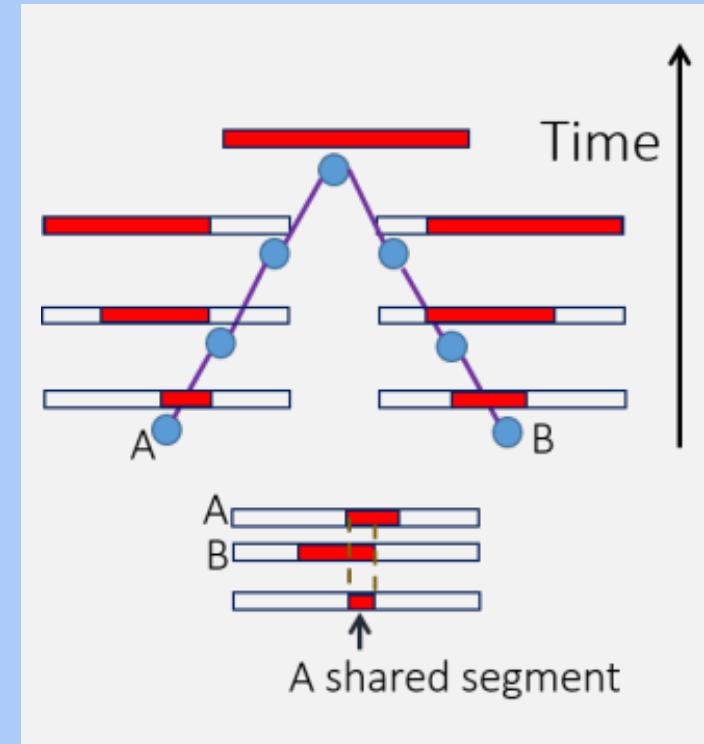
# Degree of relatedness



- Cousins:
  - $R = 3$  meioses

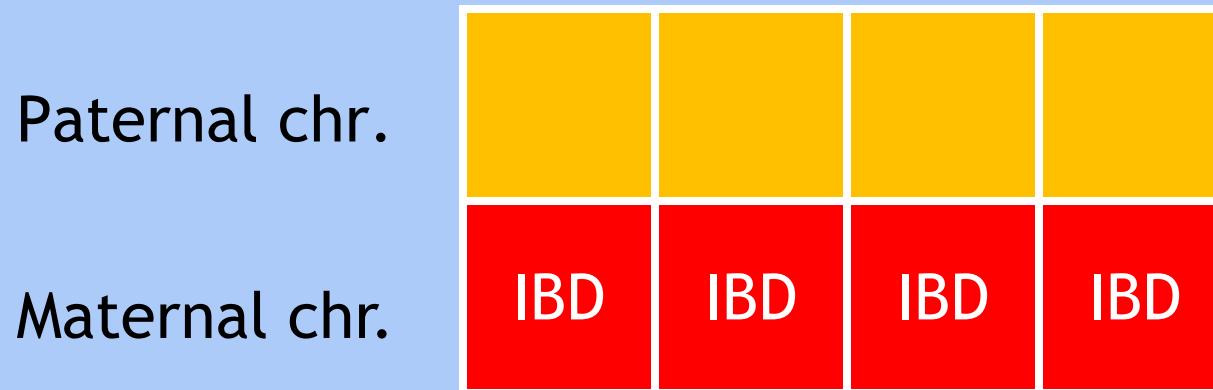
# Degree of relatedness and the fraction of the genome shared IBD

- $r = 1 / 2^R$  is the fraction of the genome shared IBD, because there is a  $1/2$  probability that the gene is passed on in each of R meioses



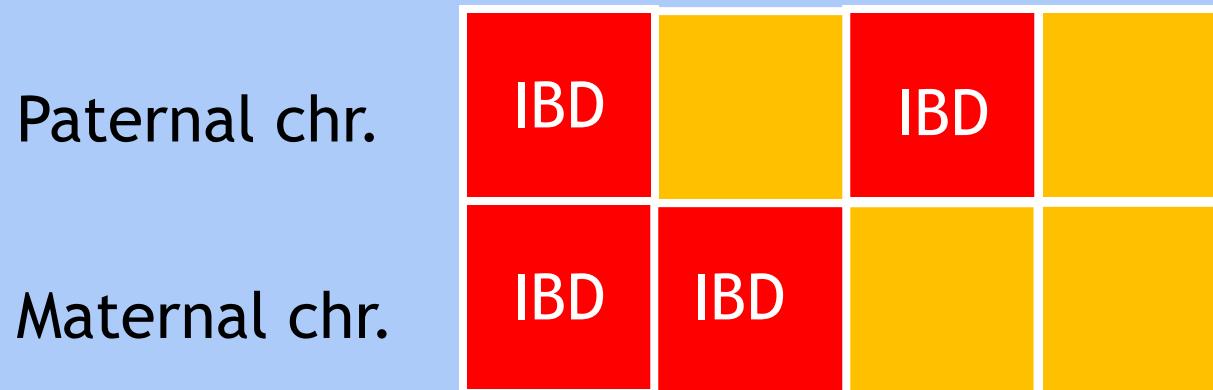
# Degree of relatedness and the fraction of the genome shared IBD

- A child shares **half** of its DNA with its parent



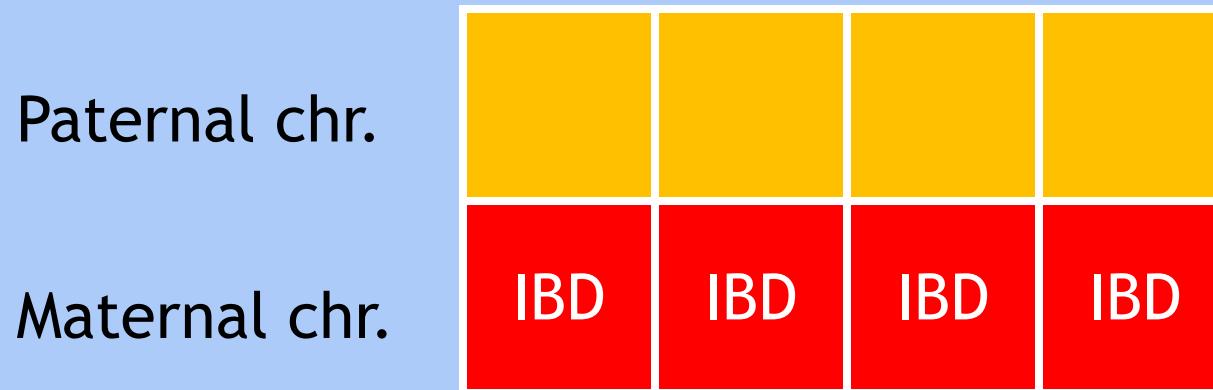
# Degree of relatedness and the fraction of the genome shared IBD

- A child shares (a different) half its DNA with its full sib



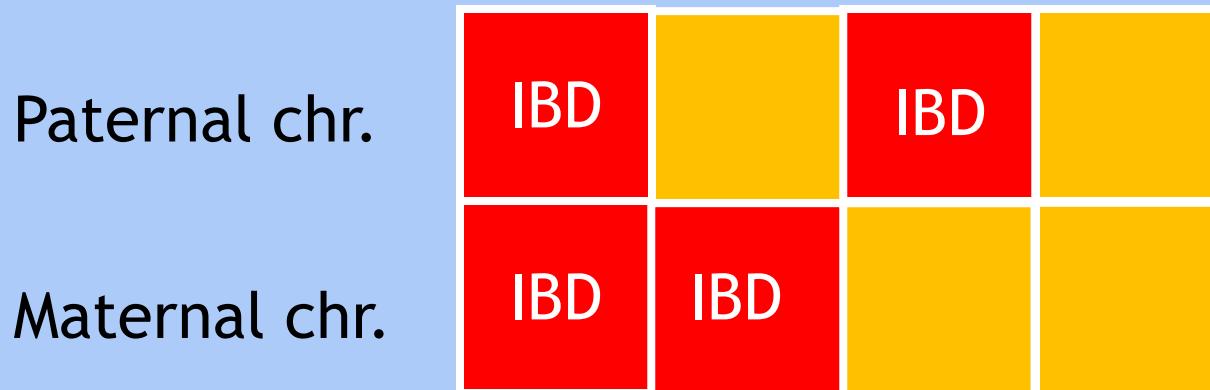
# Degree of relatedness and the fraction of the genome shared IBD

- A child has 0 probability of IBD = 0 with its parent



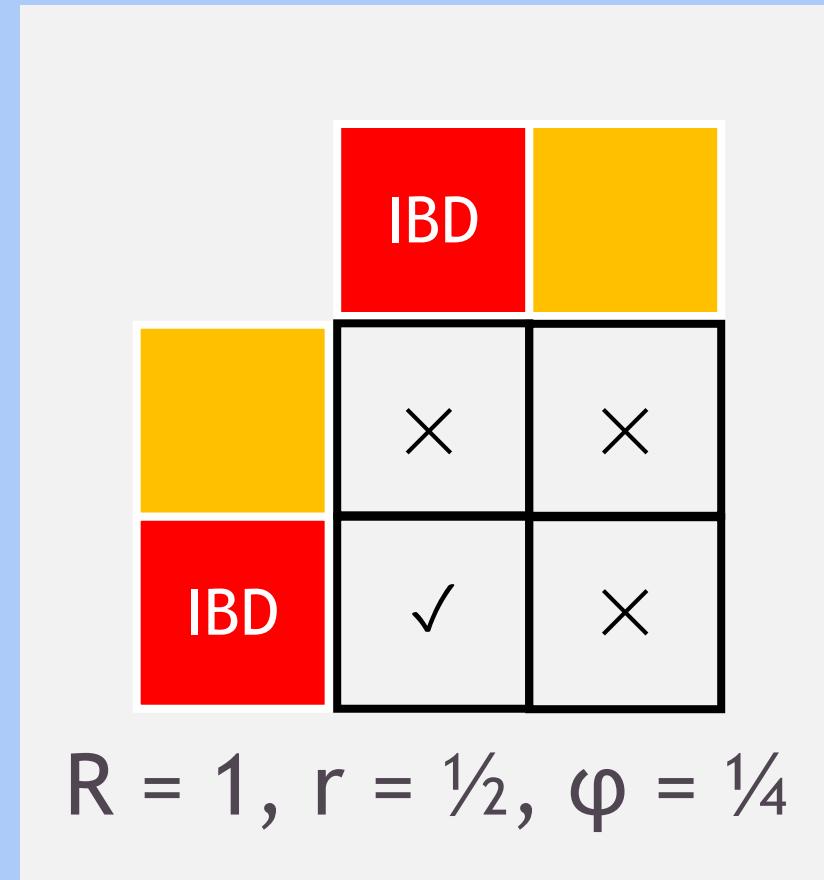
# Degree of relatedness and the fraction of the genome shared IBD

- A child has 0.25 probability of IBD = 0 with its sib



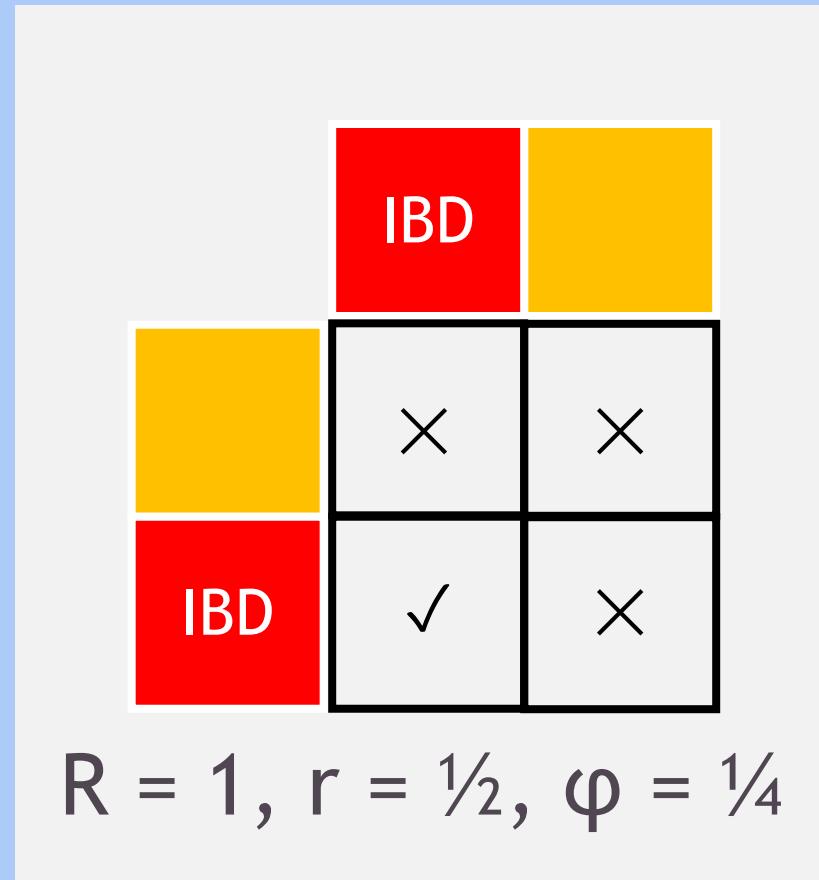
# The coefficient of relatedness $\varphi$

- $\varphi$  is the probability that any two alleles at a single locus chosen from two individuals are shared IBD



# The coefficient of relatedness $\varphi$

- $\varphi$  is equal to half of  $r = \frac{1}{2^R}$



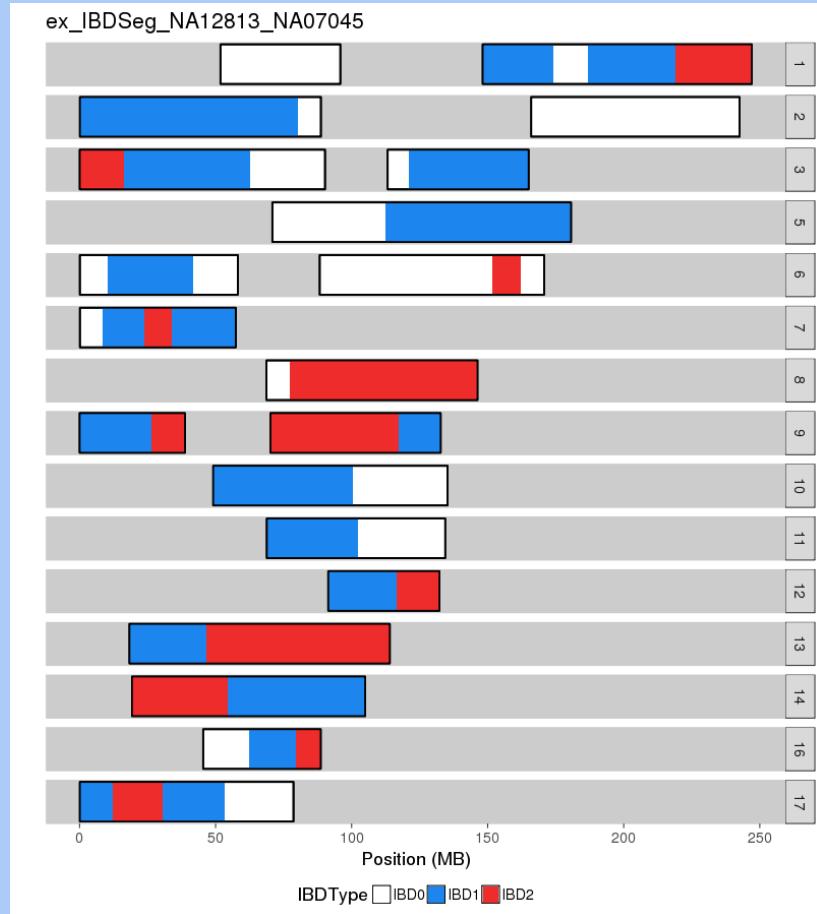
# Coefficient of relatedness and IBD = 0

- $\varphi$  decreases as the probability that a pair of individuals should be IBD = 0 increases

Relationship	R	$\varphi$	IBD = 0
Monozygotic twins	0	0.5	0
Parent-child	1	0.25	0
Full sibs	1	0.25	0.25
2 <sup>nd</sup> degree	2	0.125	0.5
3 <sup>rd</sup> degree	3	0.0625	0.75
Unrelated	$\infty$	0	1

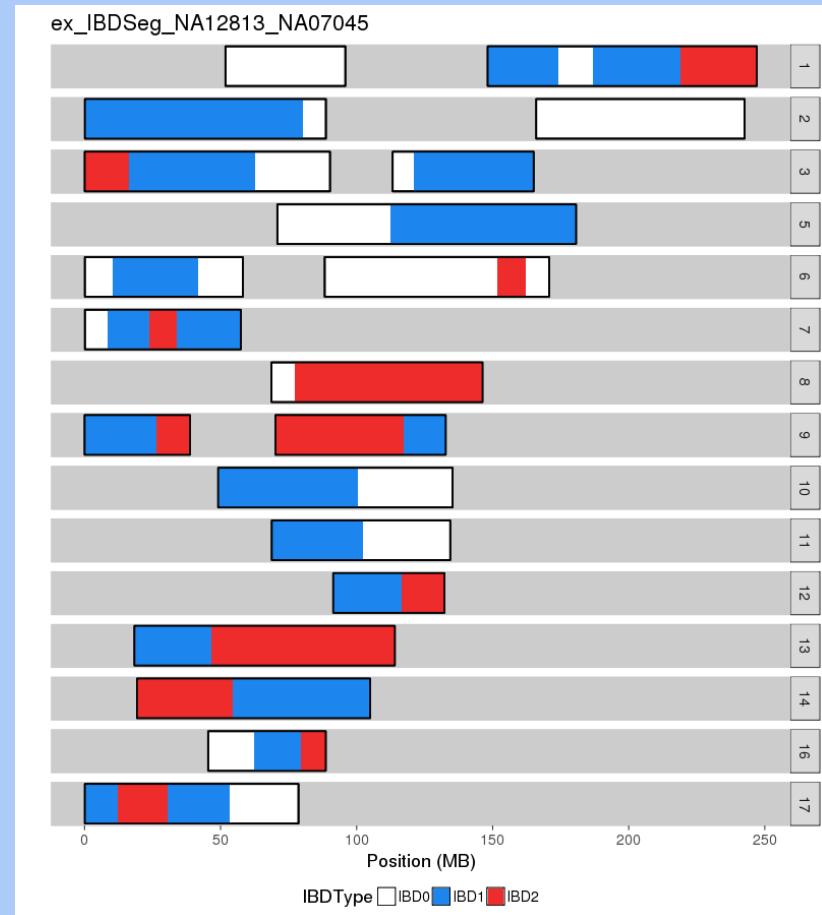
# Kinship-based Inference for GWAS (KING)

- Estimate  $\varphi$  and IBD sharing from the number of sites at which two individuals are both heterozygotes (Aa,Aa) or opposite homozygotes (AA,aa)



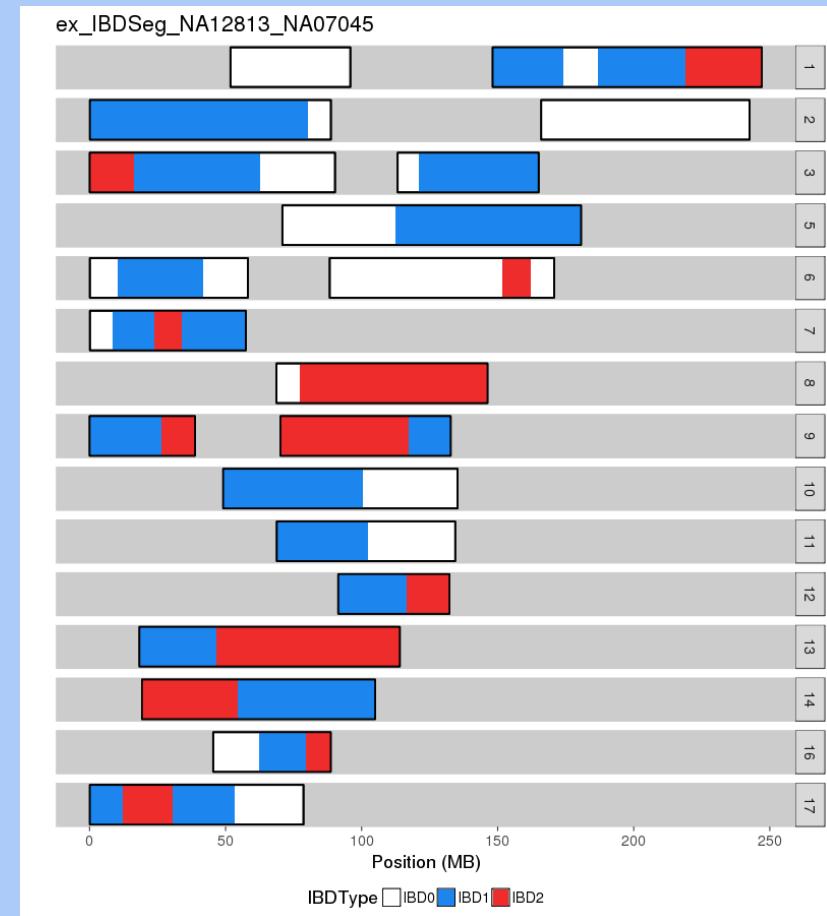
# Kinship-based Inference for GWAS (KING)

- A **robust** method that avoids estimating population allele fractions, just focuses on pairs



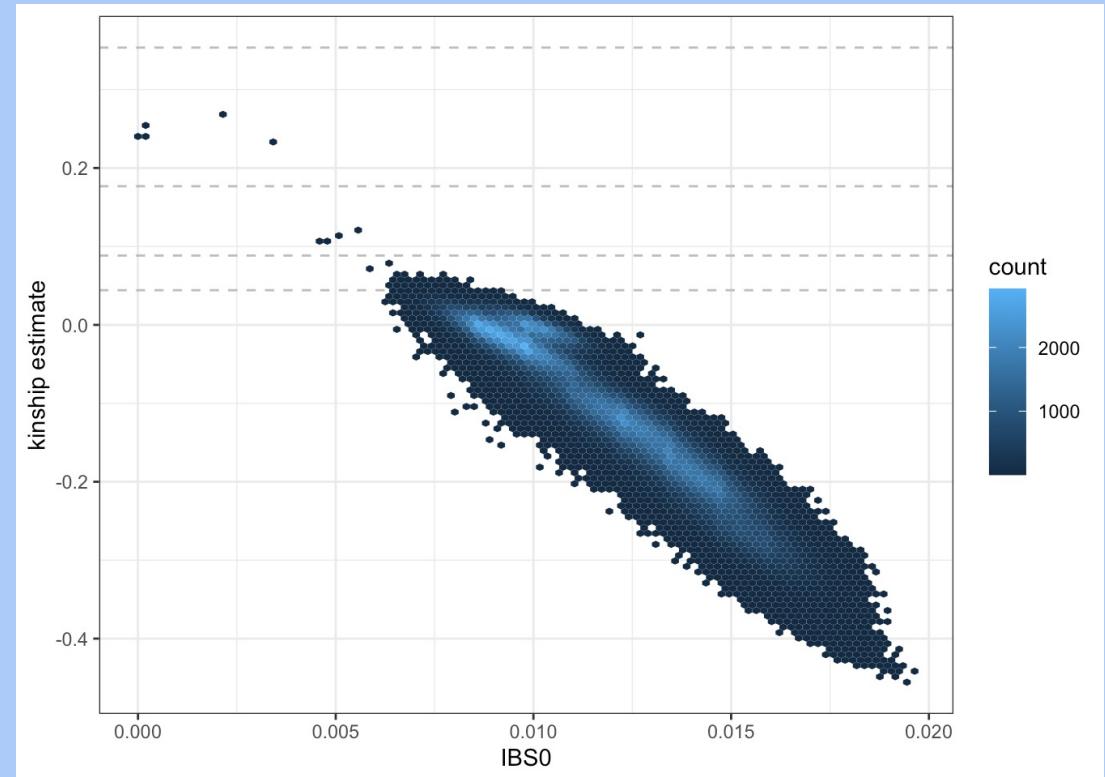
# Kinship-based Inference for GWAS (KING)

- Can generate negative estimates of  $\varphi$ , indicating individuals are from distinct populations



# Kinship-based Inference for GWAS (KING)

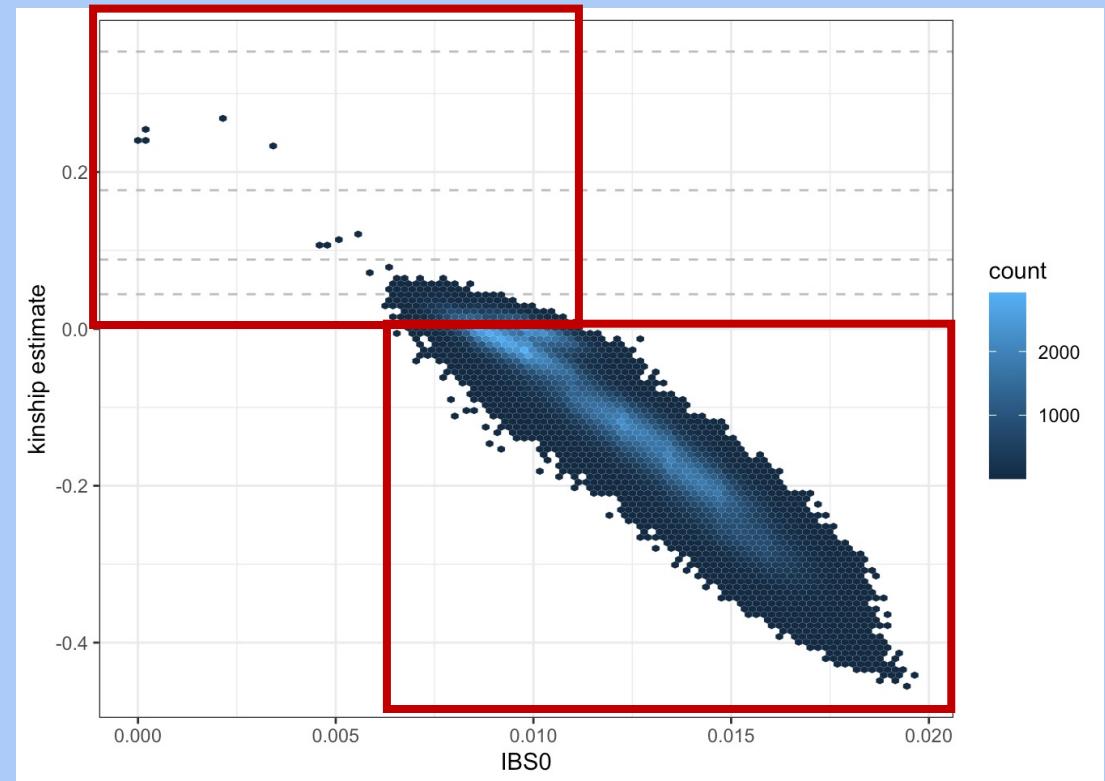
- $\varphi$  is plotted vs. the fraction of IBS = 0 sites (AA,aa)



[https://uw-gac.github.io/SISG\\_2021/ancestry-and-relatedness-inference.html](https://uw-gac.github.io/SISG_2021/ancestry-and-relatedness-inference.html)

# Kinship-based Inference for GWAS (KING)

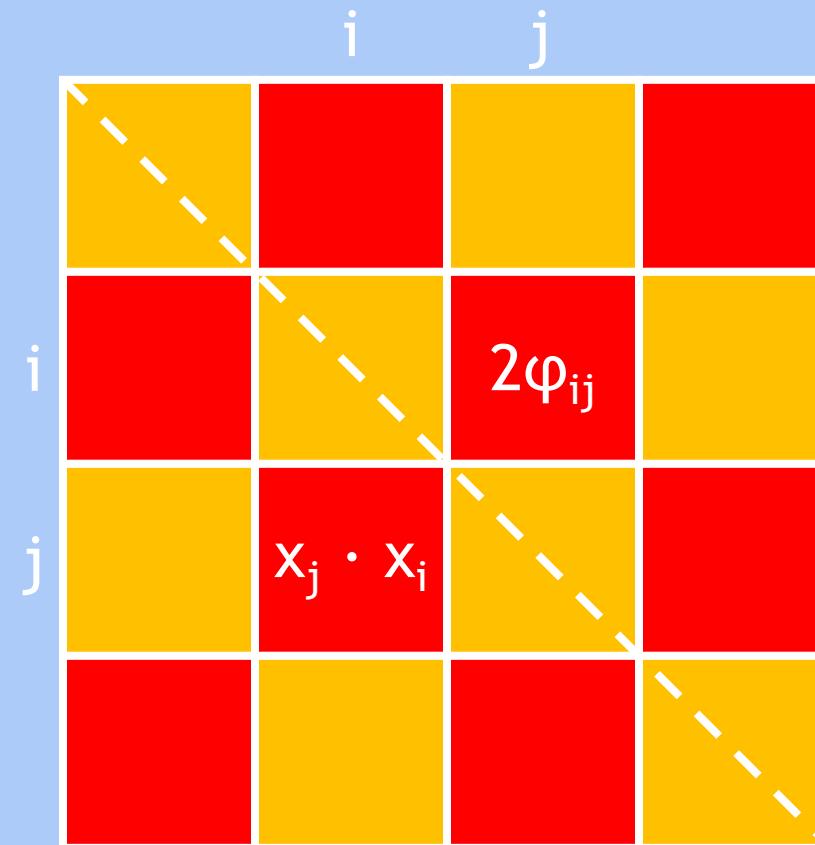
- Negative estimates indicate unrelated individuals from different populations



[https://uw-gac.github.io/SISG\\_2021/ancestry-and-relatedness-inference.html](https://uw-gac.github.io/SISG_2021/ancestry-and-relatedness-inference.html)

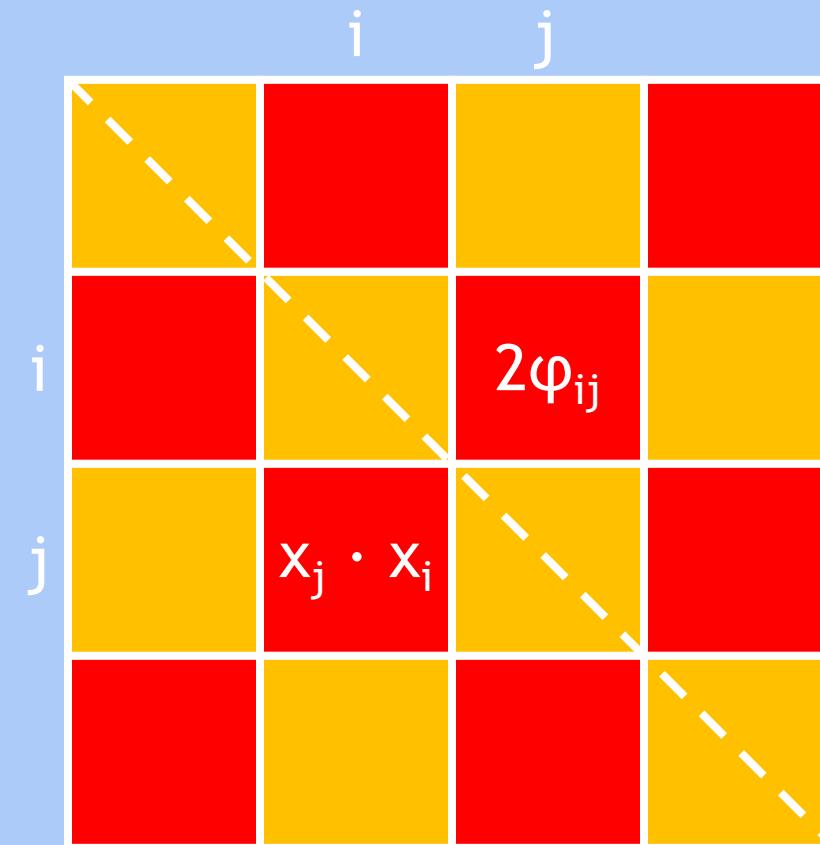
# Updating the GRM

- The KING kinship coefficients  $2\varphi$  are approximately equal to the GRM



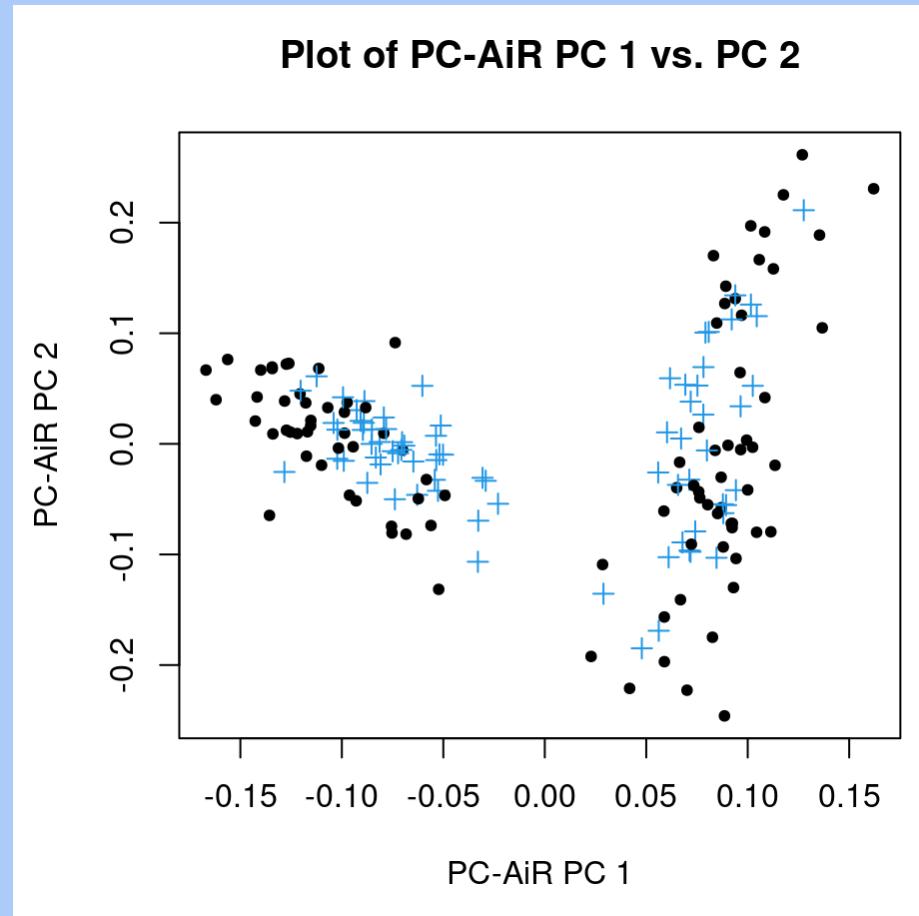
# Updating the GRM

- But the estimate may be biased by population structure



# PC-AiR: PCA in Related Samples

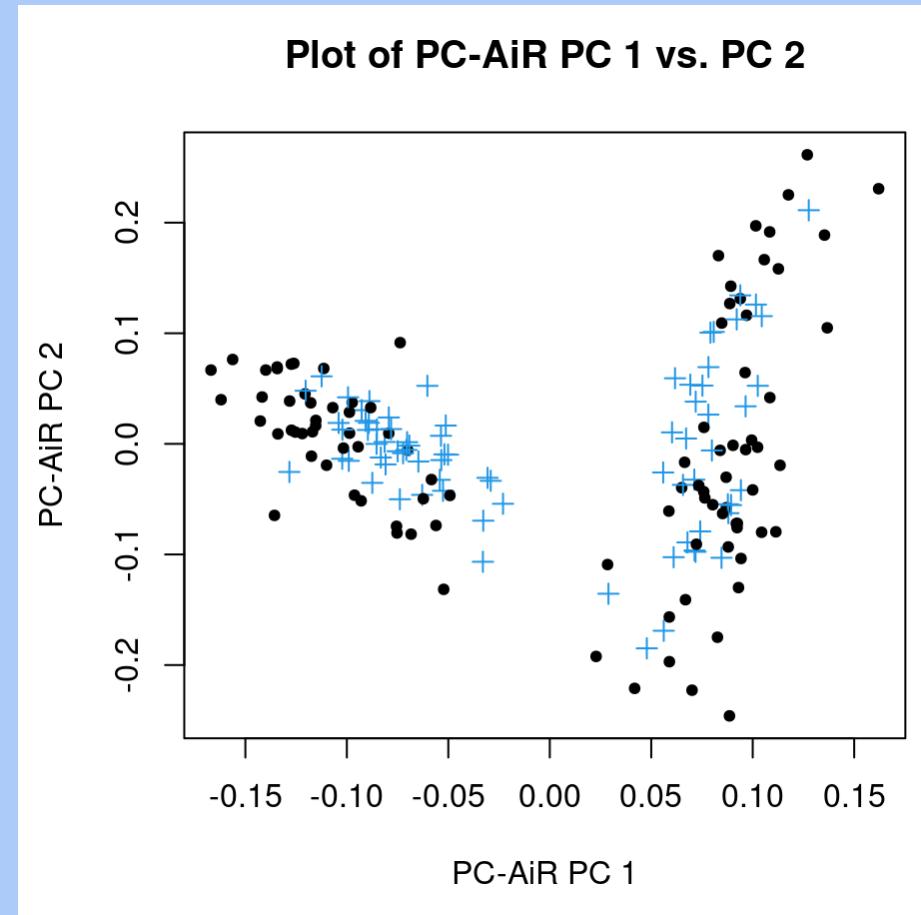
- Based on the KING estimates, PC-AiR computes PCs for a set of unrelated individuals (black)



# PC-AiR: PCA in Related Samples

- Based on the KING estimates, PC-AiR computes PCs  $\mathbf{U}$  for a set of unrelated individuals (black) with genotype matrix  $\mathbf{X}$

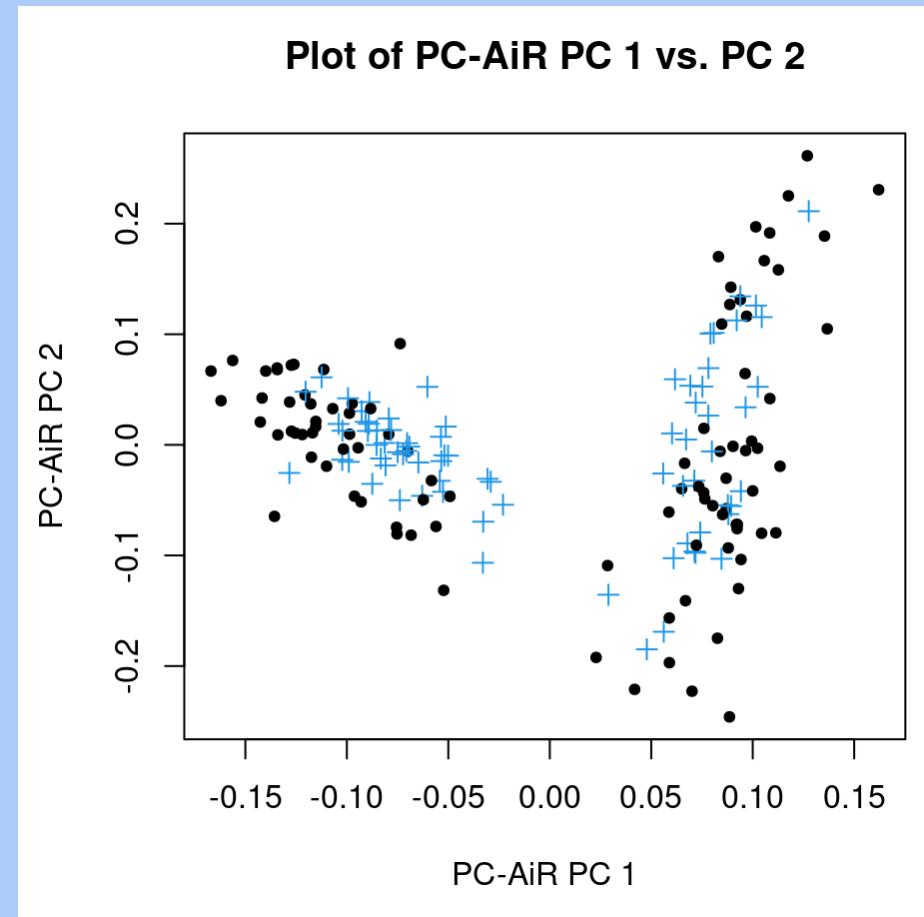
$$\mathbf{X}^T \mathbf{U} = \mathbf{V} \boldsymbol{\Sigma}$$



<https://bioconductor.org/packages-devel/bio2024/bioc/bioc/html/GENESIS/inst/doc/pcair.html>

# PC-AiR: PCA in Related Samples

- PCs for the remaining samples (blue) are imputed into the remaining subset (blue)

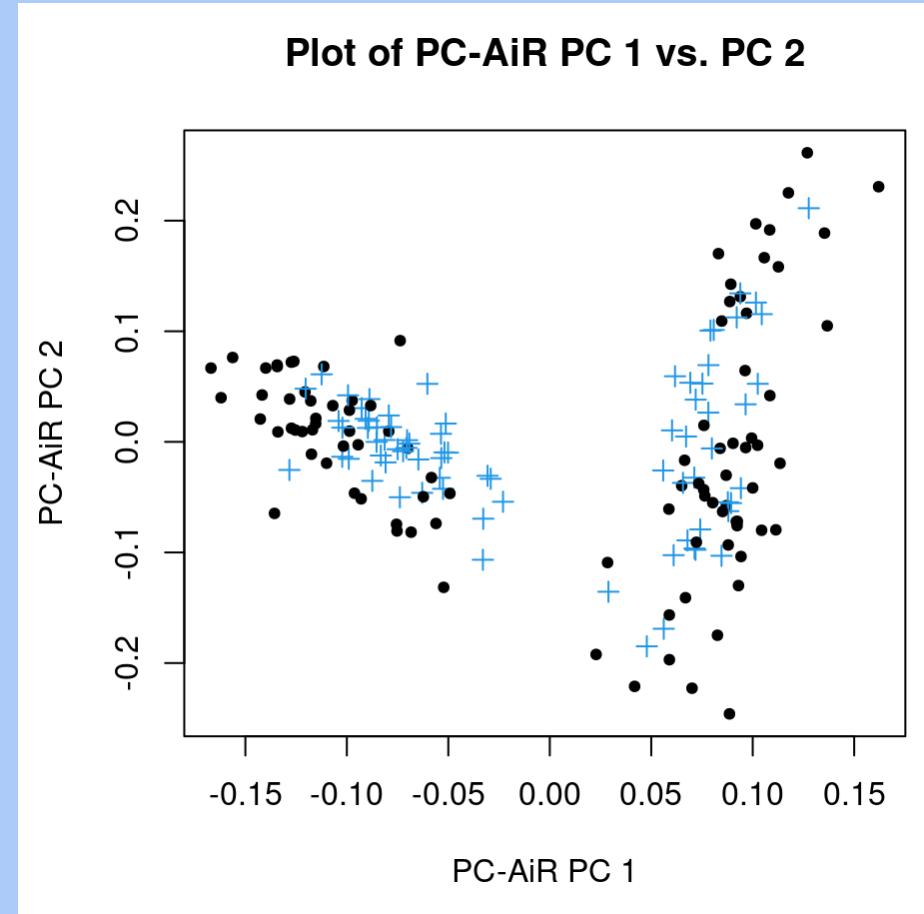


<https://bioconductor.org/packages/devel/bioc/vignettes/GENESIS/inst/doc/pcair.html>

# PC-AiR: PCA in Related Samples

- PCs  $\mathbf{U}'$  for the remaining samples (blue) with genotype matrix  $\mathbf{X}'$  are imputed into the remaining subset (black)

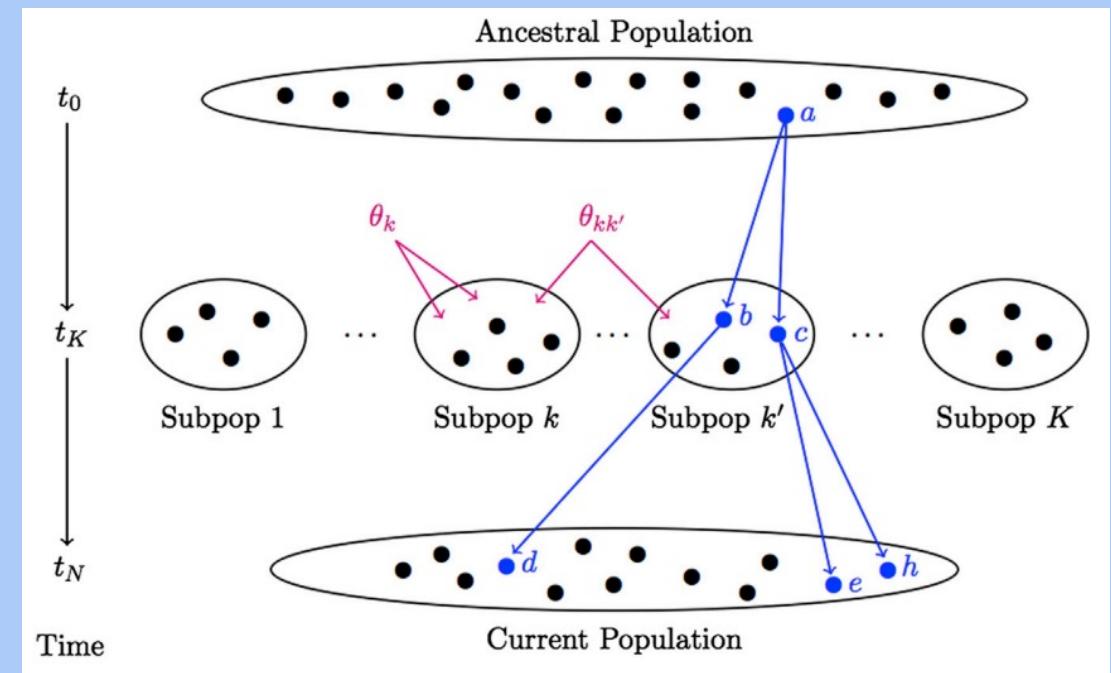
$$\mathbf{X}' (\mathbf{X}^T \mathbf{U} \Sigma^{-1}) = \mathbf{X}' \mathbf{V}$$



<https://bioconductor.org/packages/devel/bioc/vignettes/GENESIS/inst/doc/pcair.html>

# PC-Relate

- PC-Relate uses the updated PCs to distinguish shared genetic ancestry from recent common ancestors



<https://pubmed.ncbi.nlm.nih.gov/26748516/>

# PC-Relate

- Each individual's “best-fit” genotype is predicted from its PCs

$$\mathbb{E} (g_{ik} \mid u_{ij}) = 2p_k + 2p_k (1 - p_k) u_{ij} \lambda_{jj} \cdot v_{kj}$$

# PC-Relate

- Each individual's “best-fit” genotype is predicted from its PCs

$$\mathbb{E} (g_{ik} \mid u_{ij}) = 2p_k + 2p_k (1 - p_k) u_{ij} \lambda_{jj} \cdot v_{kj}$$

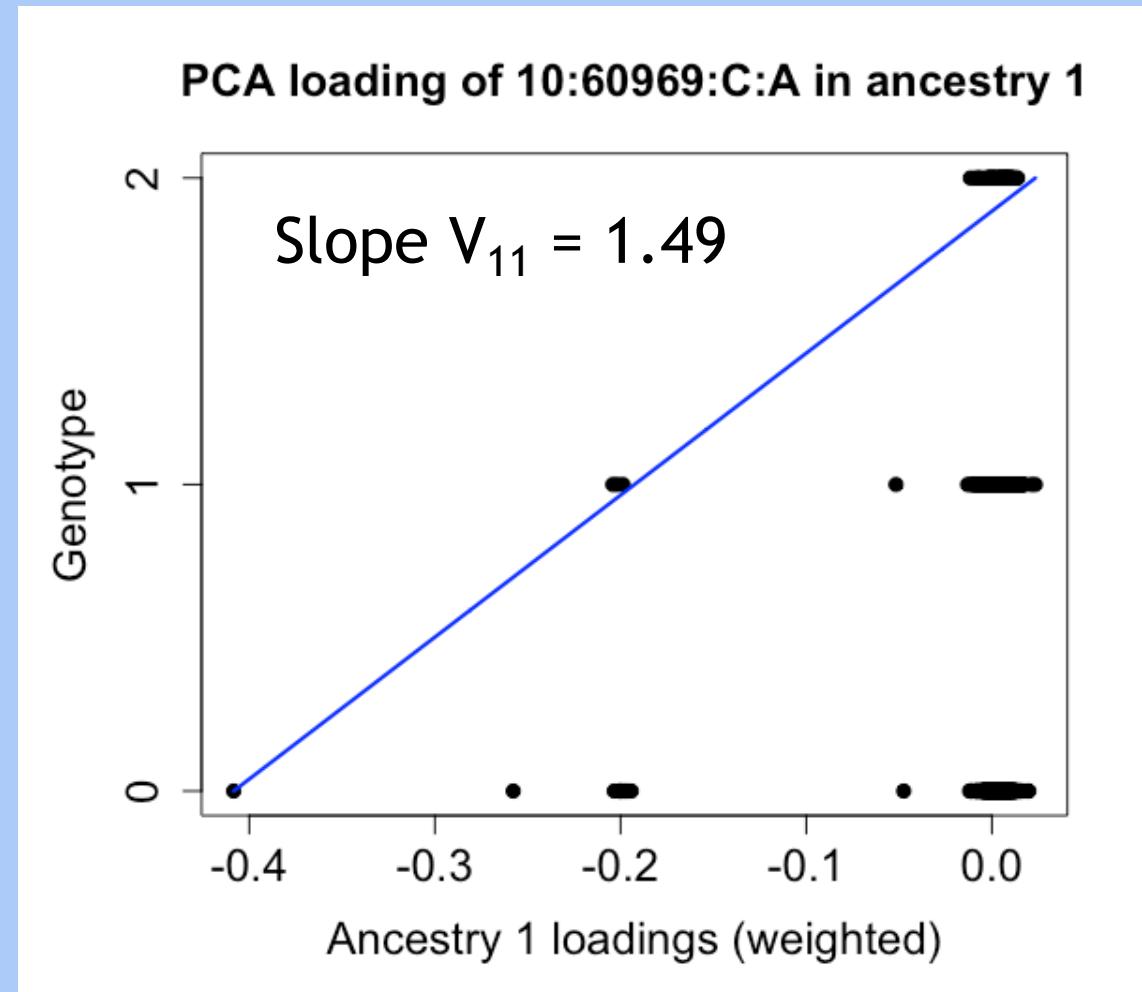
Population allele  
frequency

Amount of  
ancestry j

SNP k genotype in  
ancestry j

# PC-Relate

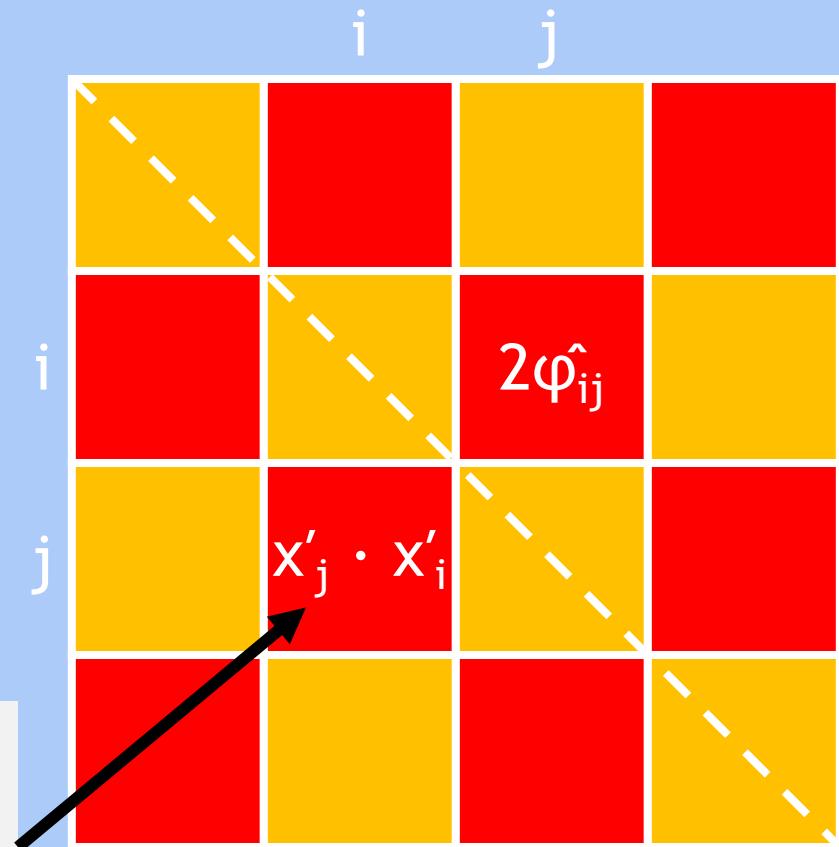
- The slope of the best fit line of genotype vs. (weighted) PC1 is equal to the expected SNP genotype in ancestry 1



# PC-Relate

- An updated GRM that reflects only recent common ancestry can be constructed using the “best-fit” genotypes  $2p_{ik}$  for each individual  $i$  at SNP  $k$

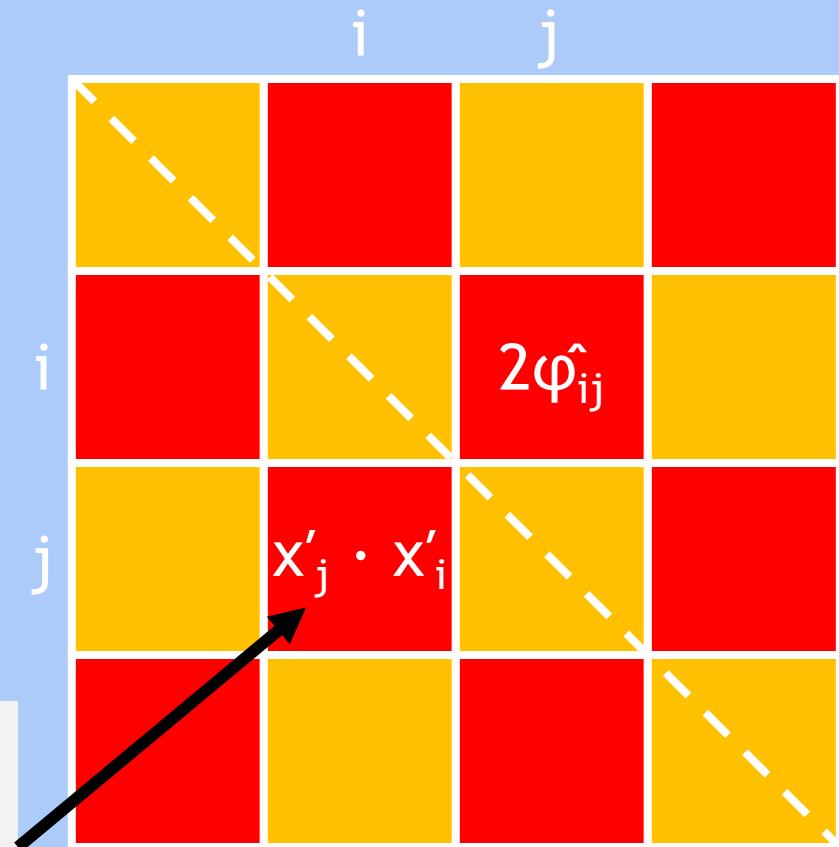
$$2\hat{\varphi}_{ij} = \frac{\sum_k (g_{ik} - 2p_{ik})(g_{jk} - 2p_{jk})}{2\sqrt{p_{ik}(1-p_{ik})p_{jk}(1-p_{jk})}}$$



# PC-Relate

- The updated GRM will be used for fitting a generalized linear model during association testing

$$2\hat{\varphi}_{ij} = \frac{\sum_k (g_{ik} - 2p_{ik})(g_{jk} - 2p_{jk})}{2\sqrt{p_{ik}(1-p_{ik})p_{jk}(1-p_{jk})}}$$

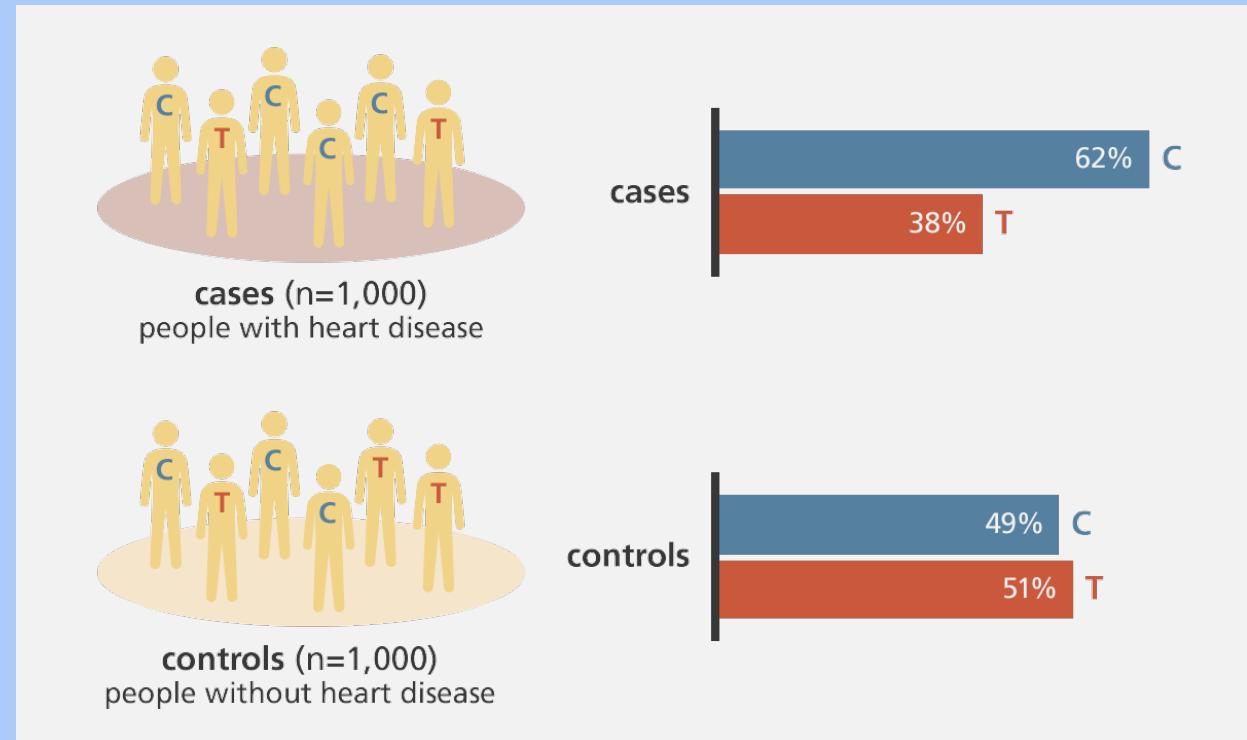


# Association testing

## Logistic regression and linear mixed models

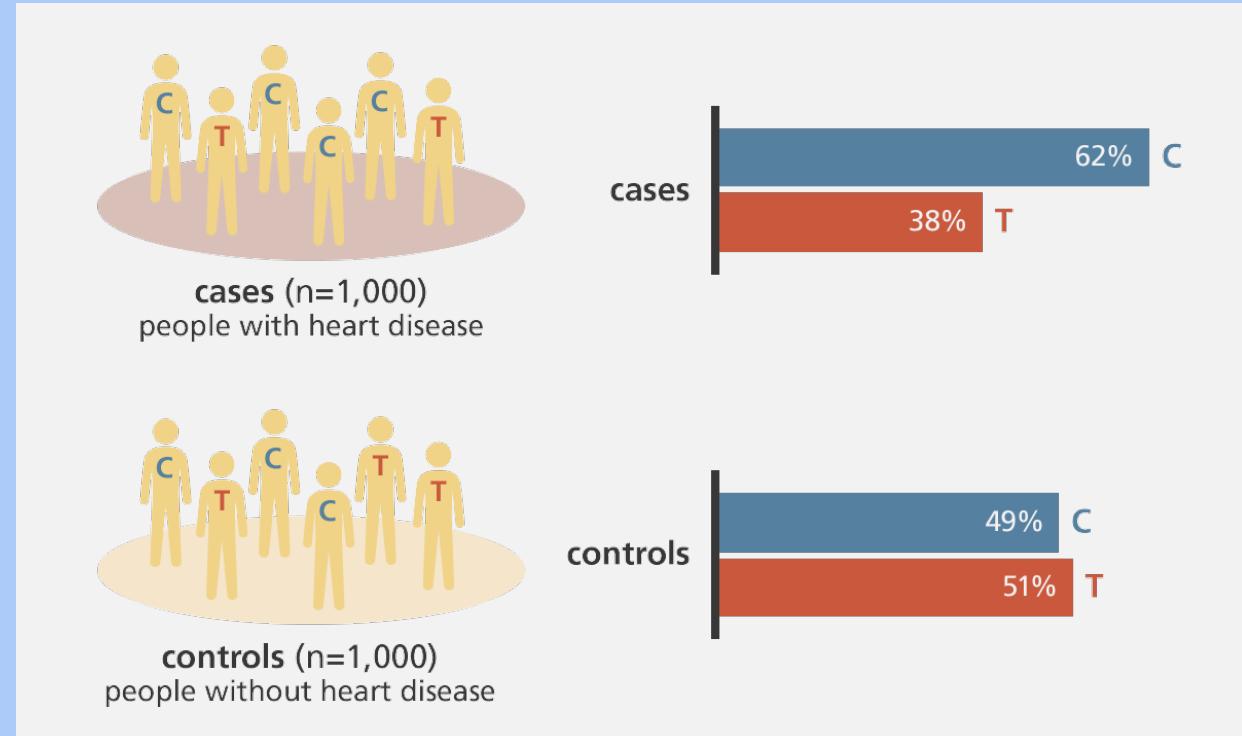
# Case-control studies

- Is a genetic variant associated with disease?



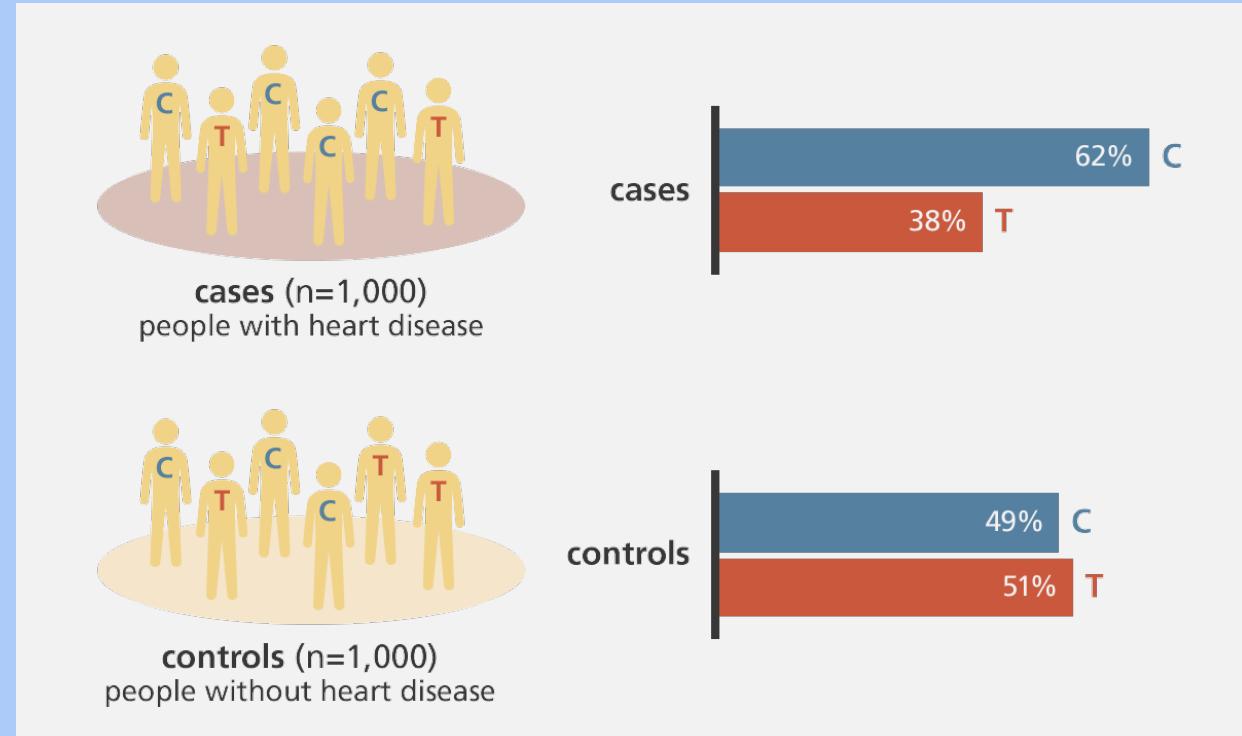
# Case-control studies

- Is a genetic variant enriched in people with disease compared to people without?



# Case-control studies

- To find out, collect many people with disease (Cases) and many healthy individuals (Controls) from the same population



# The odds ratio

- The OR is the ratio of the odds that Cases have the risk allele ( $a / c$ ) to the odds that Controls have the risk allele ( $b / d$ )

	Cases	Controls
C	a	b
T	c	d

$$OR = (a / c) / (b / d) = (ad) / (bc)$$

# The odds ratio

- The OR is the ratio of the odds that Cases have the risk allele ( $620 / 380$ ) to the odds that Controls have the risk allele ( $490 / 510$ )

	Cases	Controls
C	620	490
T	380	510

$$OR = (620 \times 510) / (490 \times 380) = 1.70$$

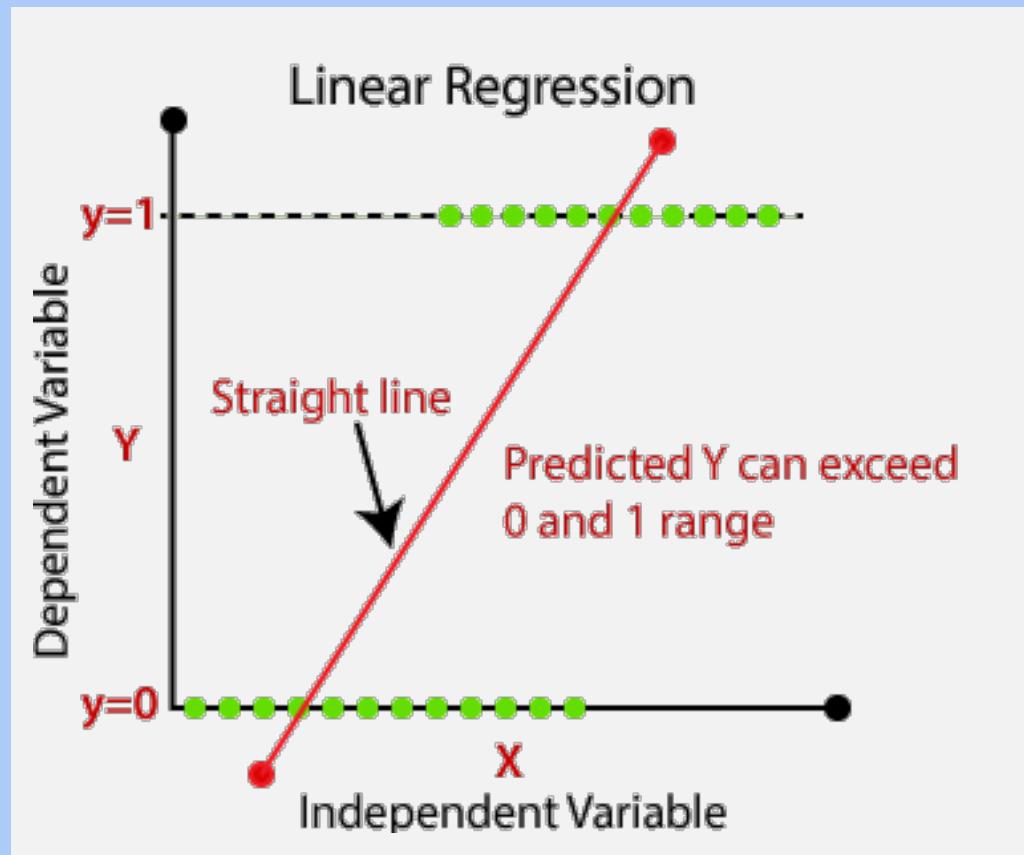
# The odds ratio

- The OR is a **crude** measure of association that is not **adjusted** for other covariates (age, sex, ethnicity, etc.) that may also be associated with disease

	Cases	Controls
C	620	490
T	380	510

$$OR = (620 \times 510) / (490 \times 380) = 1.70$$

# Linear vs. logistic regression



- In linear regression, we can find the association of a **continuous variate**  $Y$  with a predictor  $X_1$  and other covariates  $X_2, X_3$ , etc.

# Linear vs. logistic regression

- Best estimate of the slope of Y vs. X

$$\hat{\beta} = \frac{\sum_i (Y_i - \bar{Y}) (X_i - \bar{X})}{\sum_i (X_i - \bar{X})^2}$$

# Linear vs. logistic regression

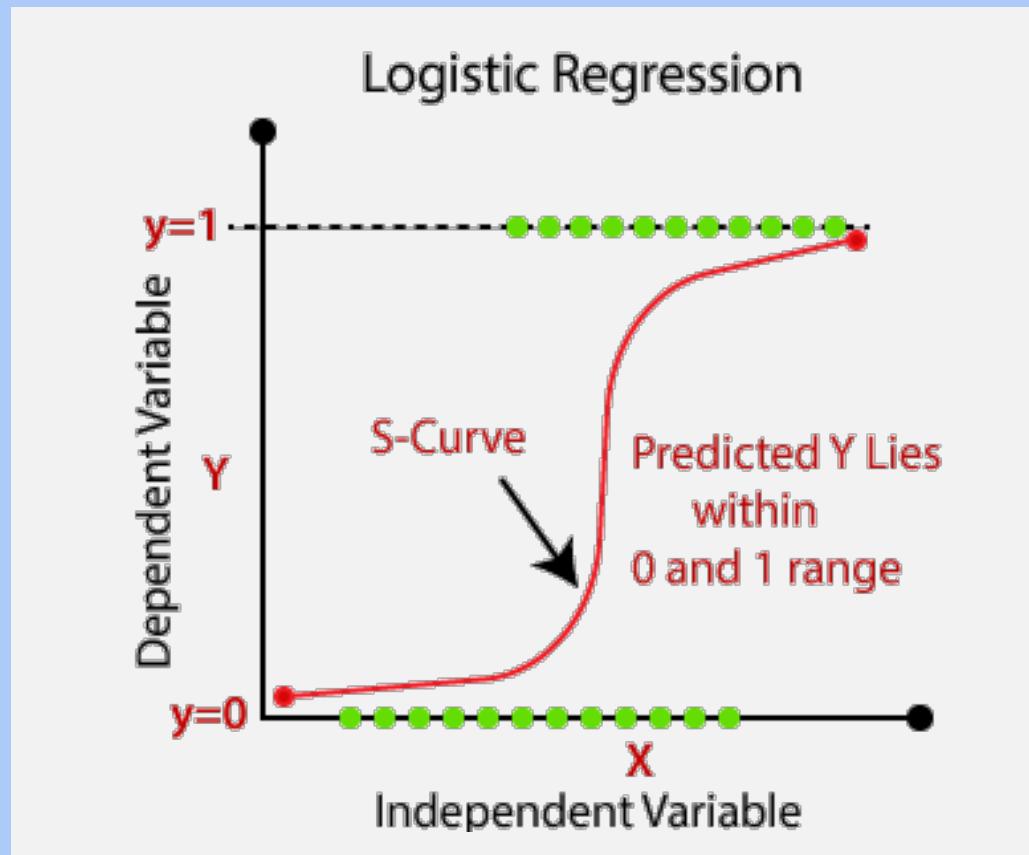
- Standard error of the estimate

$$s_{\hat{\beta}} = \sqrt{\frac{\sum_i (Y_i - \hat{\alpha} - \hat{\beta}X_i)^2 / (N - 2)}{\sum_i (X_i - \bar{X})^2}}$$

# Linear vs. logistic regression

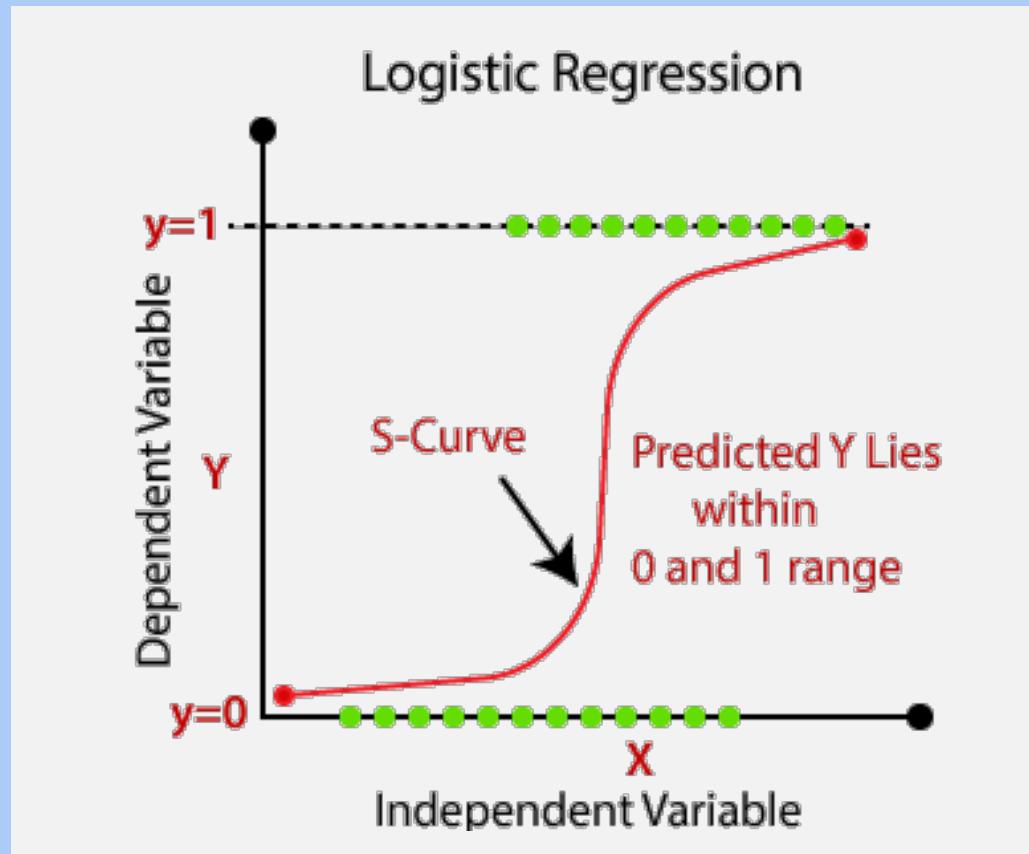
- Using the t-test, we can find out if  $\hat{\beta} / s$  is statistically significantly different from 0

# Linear vs. logistic regression



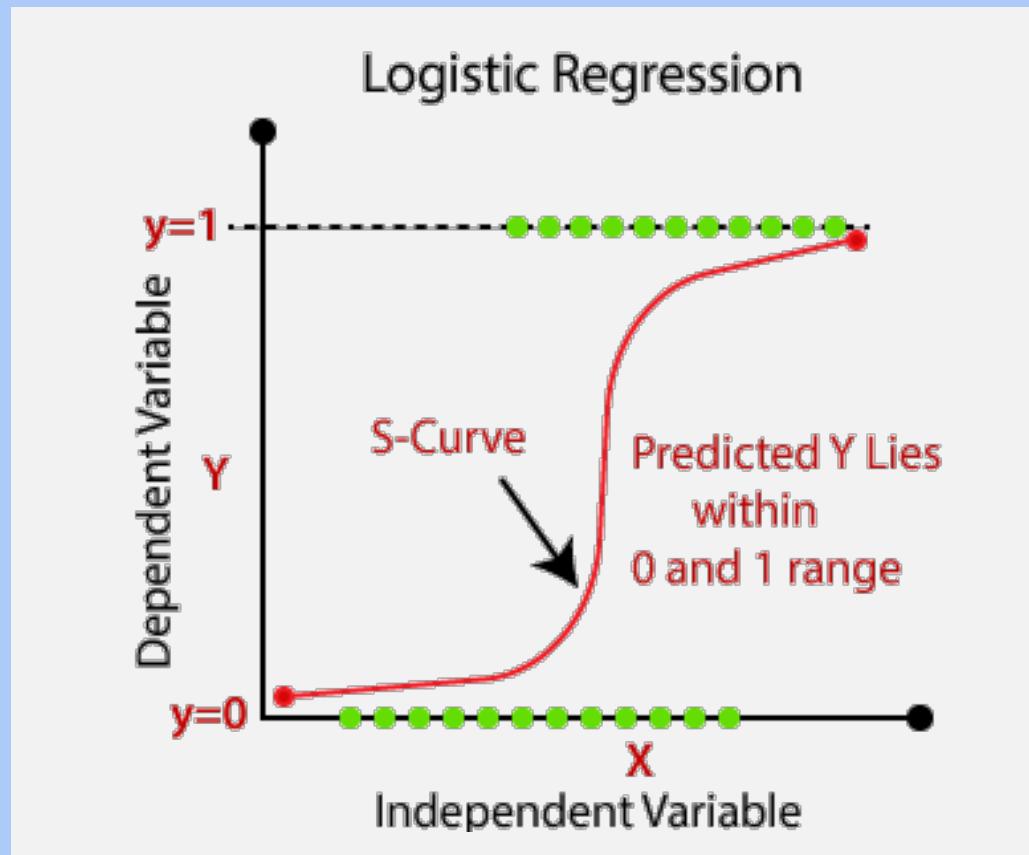
- In logistic regression, we can find the association of a **binary variate**  $Y$  with a predictor  $X_1$  and other covariates  $X_2, X_3$ , etc.

# Linear vs. logistic regression



- The sigmoid curve is an individual's probability of developing disease

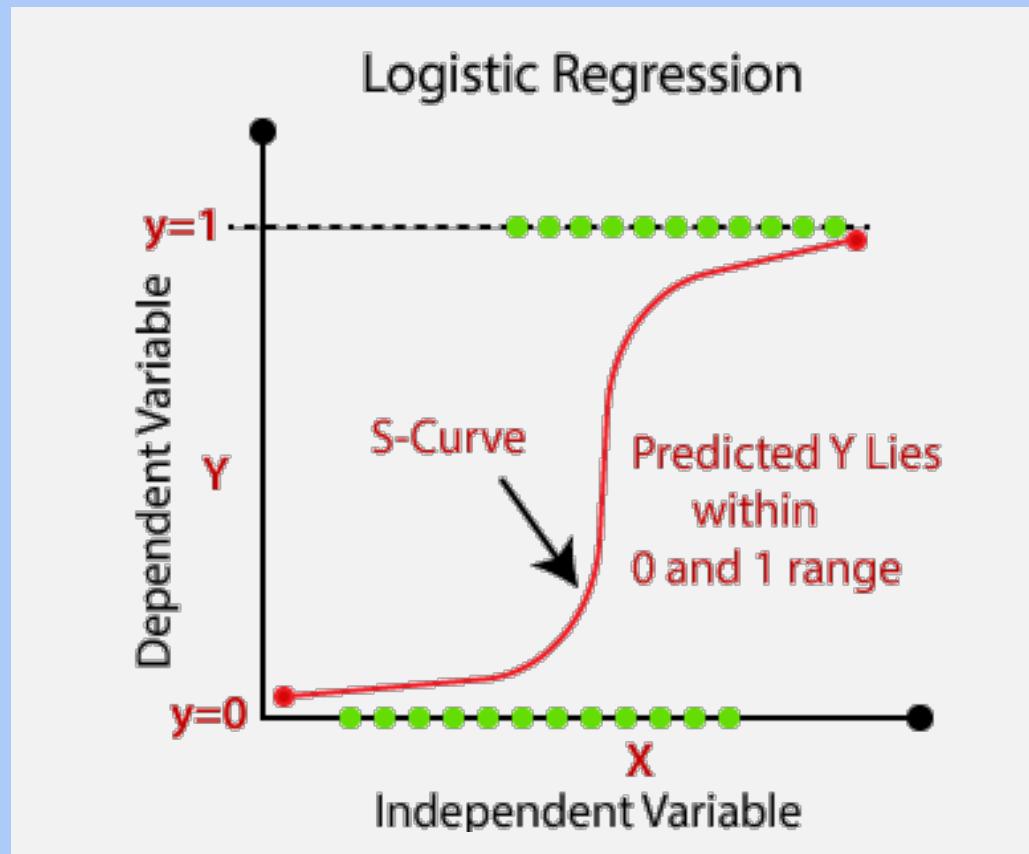
# Linear vs. logistic regression



- The logistic model describes an individual's unobserved disease risk

$$p = \frac{e^{\beta_0 + X_1 \beta_1}}{1 + e^{\beta_0 + X_1 \beta_1}}$$

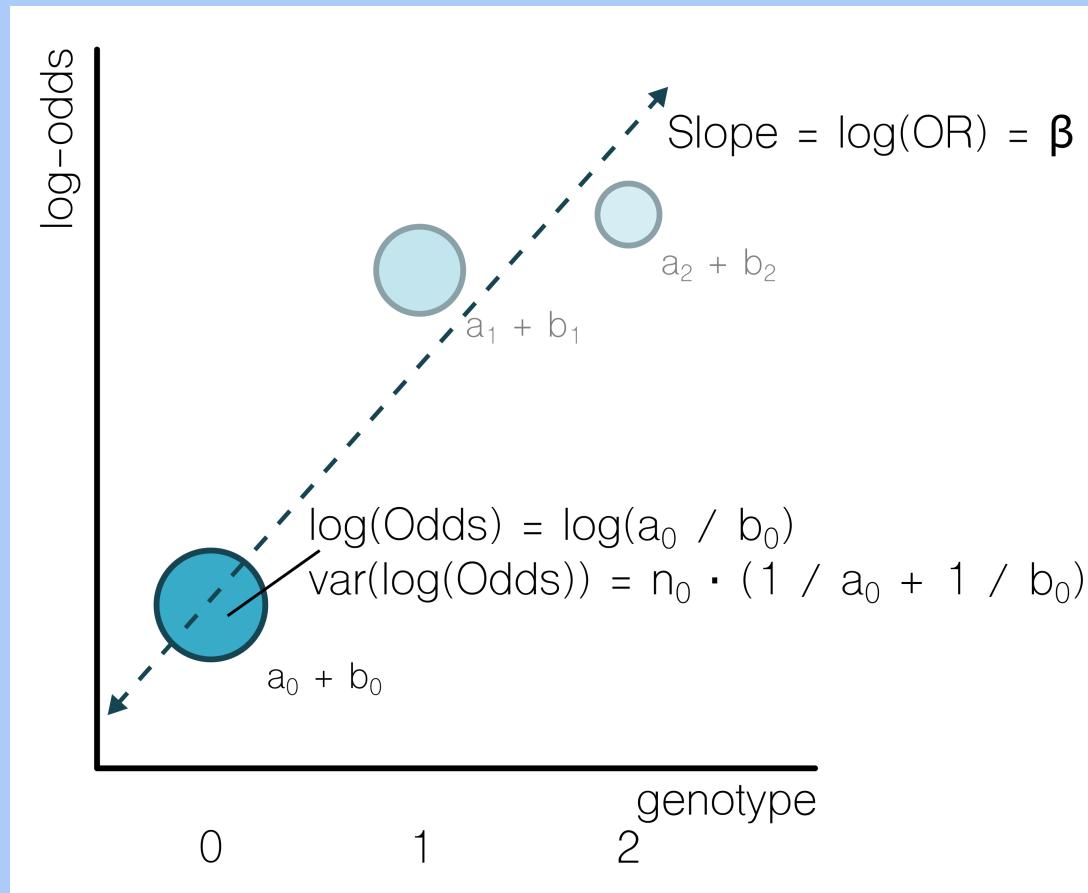
# Linear vs. logistic regression



- The **logit** function turns this problem into one of linear regression

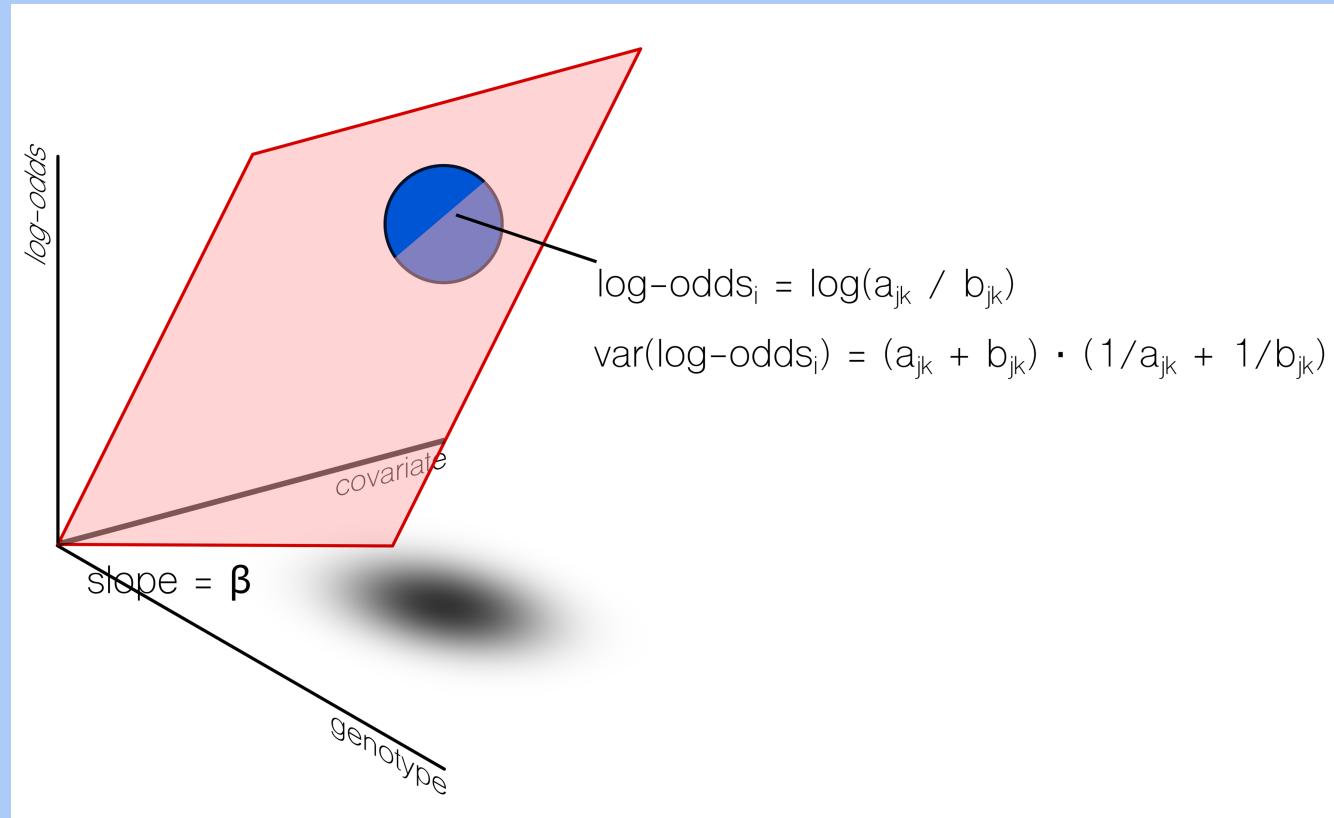
$$\log \frac{p}{1-p} = \beta_0 + X_1\beta_1$$

# Linear vs. logistic regression



- Logistic regression can be thought of as linear regression is we transform the OR into the log(OR), and regress vs. SNP genotype

# Linear vs. logistic regression



- Other covariates can be accounted for as additional independent variables

# Linear vs. logistic regression

- The model is actually fit using the principle of **maximum-likelihood**

# Linear vs. logistic regression

- $Y_i$  is a binary indicator of disease for individual  $i$ , and  $p_i$  is the unobserved (conditional) probability of disease

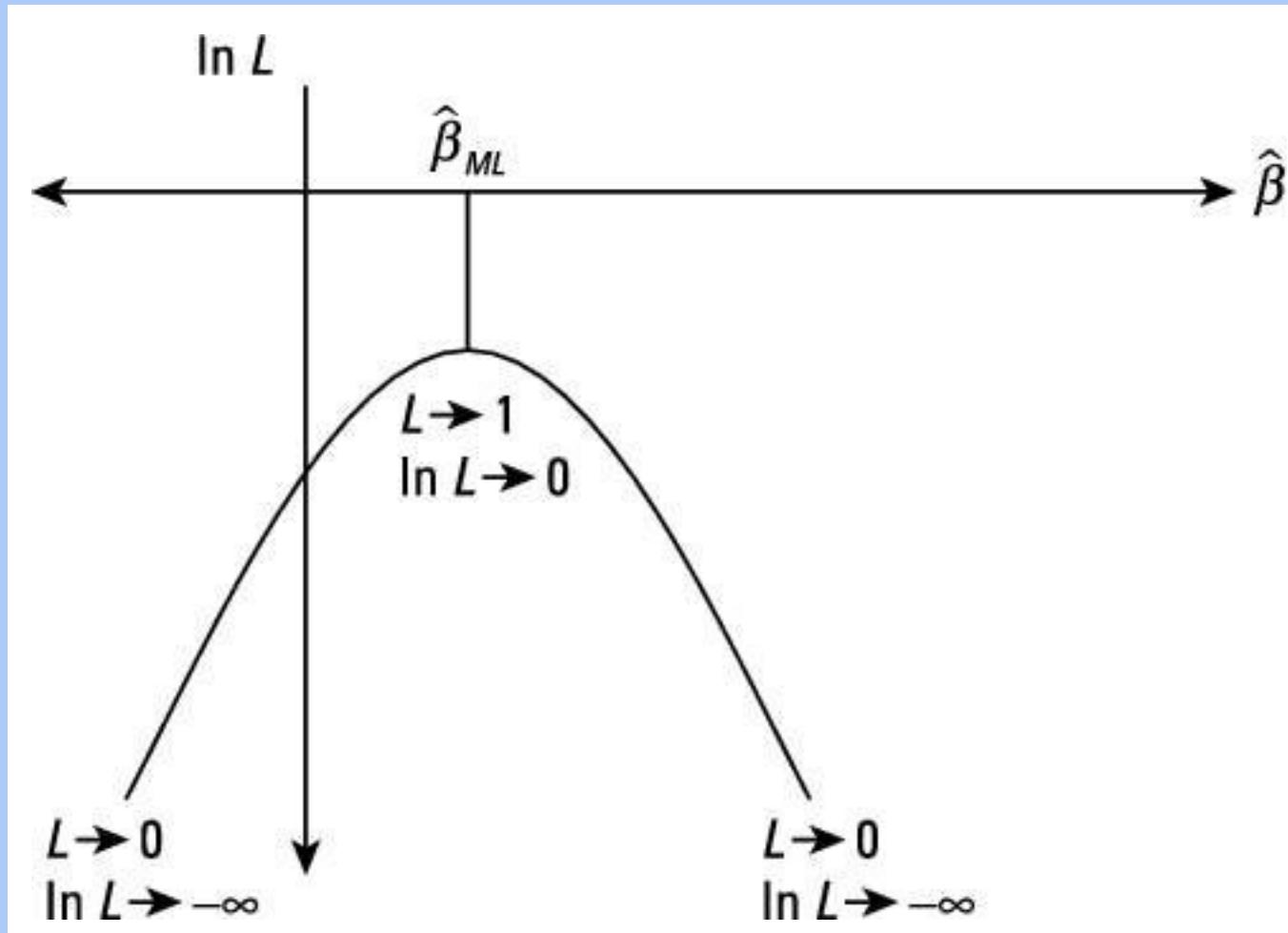
$$\mathcal{L} = \prod_i p_i^{y_i} (1 - p_i)^{1-y_i}$$

# Linear vs. logistic regression

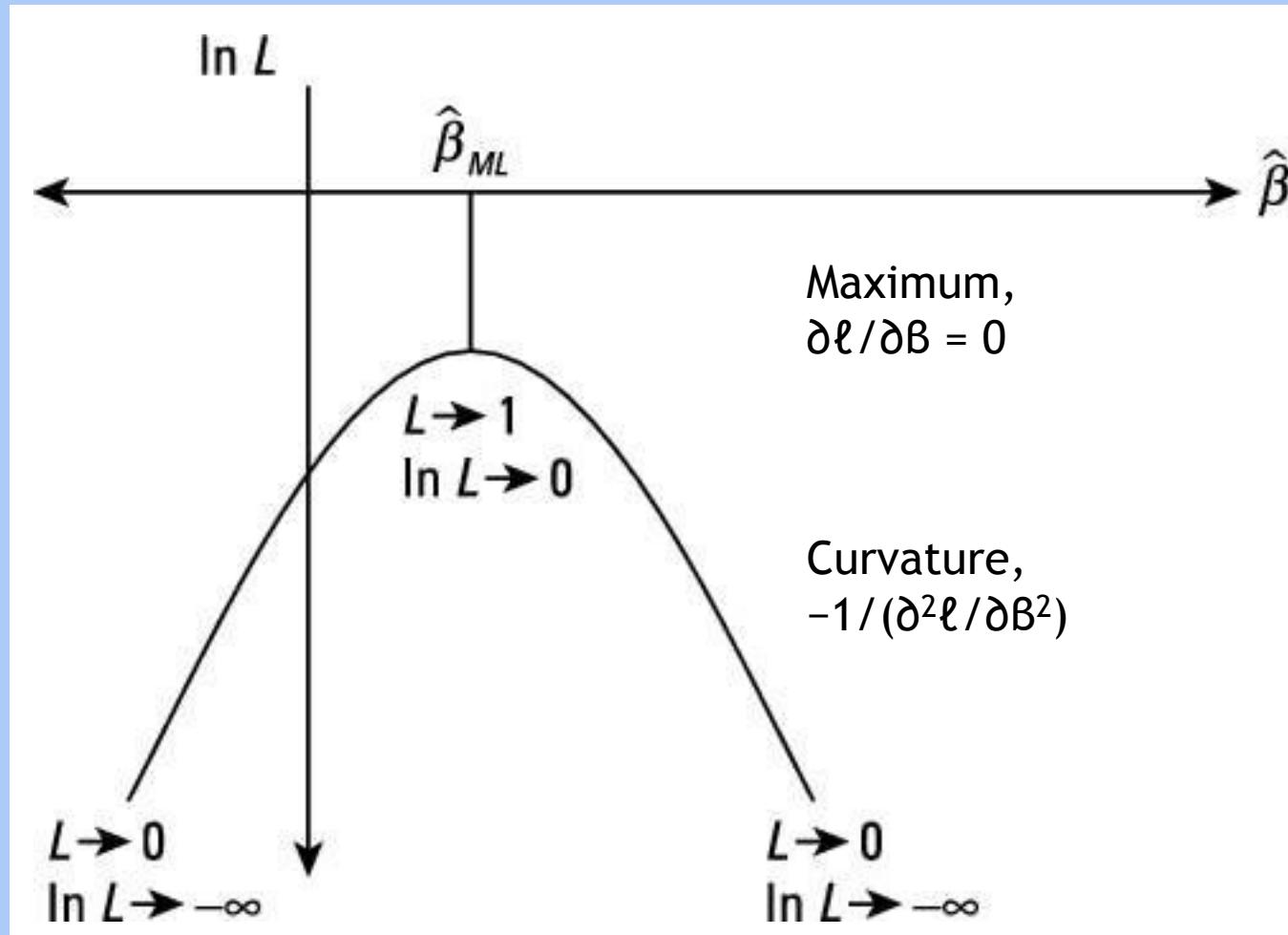
- The **log-likelihood** is a convex function of the parameters  $\beta$  which we can maximize

$$\ell = \sum_i y_i (\beta_0 + X_1 \beta_1) + \log (1 + e^{\beta_0 + X_1 \beta_1})$$

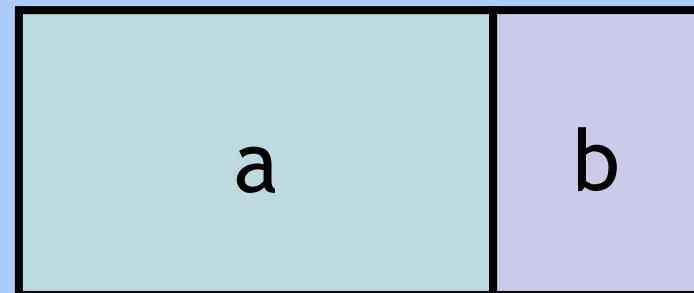
# Linear vs. logistic regression



# Linear vs. logistic regression



# Simulating a binary phenotype

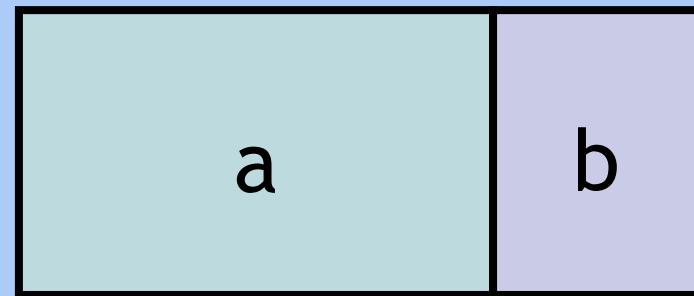


- If odds =  $a / b$ , then prob =  $a / (a + b) = \text{odds} / (1 + \text{odds})$

# Simulating a binary phenotype

$$\log(\text{odds}) = \beta_0 + X_1\beta_1$$

- $\beta_0$  is the baseline odds
- $\beta_1$  is the log-OR
- $X_1$  is the SNP genotype

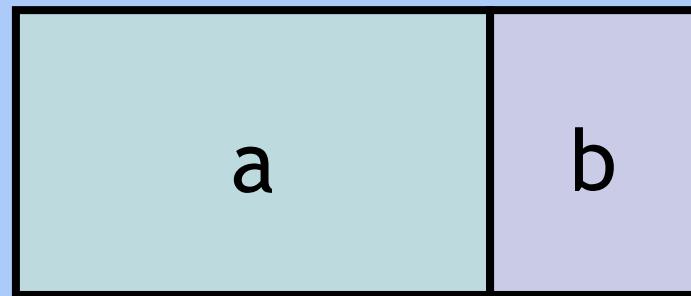


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

# Simulating a binary phenotype

$$\text{prob} = \frac{e^{\beta_0 + X_1 \beta_1}}{1 + e^{\beta_0 + X_1 \beta_1}}$$

- prob is the probability of developing disease (being a Case in the study)

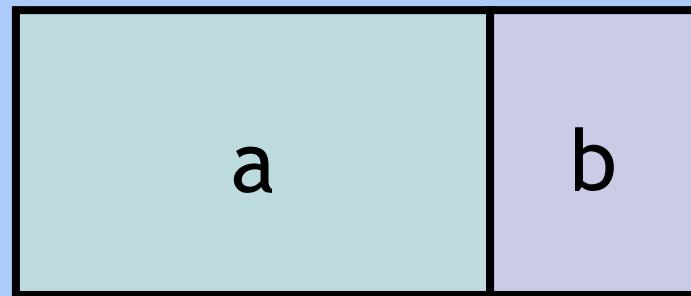


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

# Simulating a binary phenotype

$$\text{prob} = \frac{e^{(X_1 - \bar{X}_1)\beta_1}}{1 + e^{(X_1 - \bar{X}_1)\beta_1}}$$

- $\beta_0$  becomes the mean log-odds, so that the mean odds of disease is 1 (50% Cases, 50% Controls)

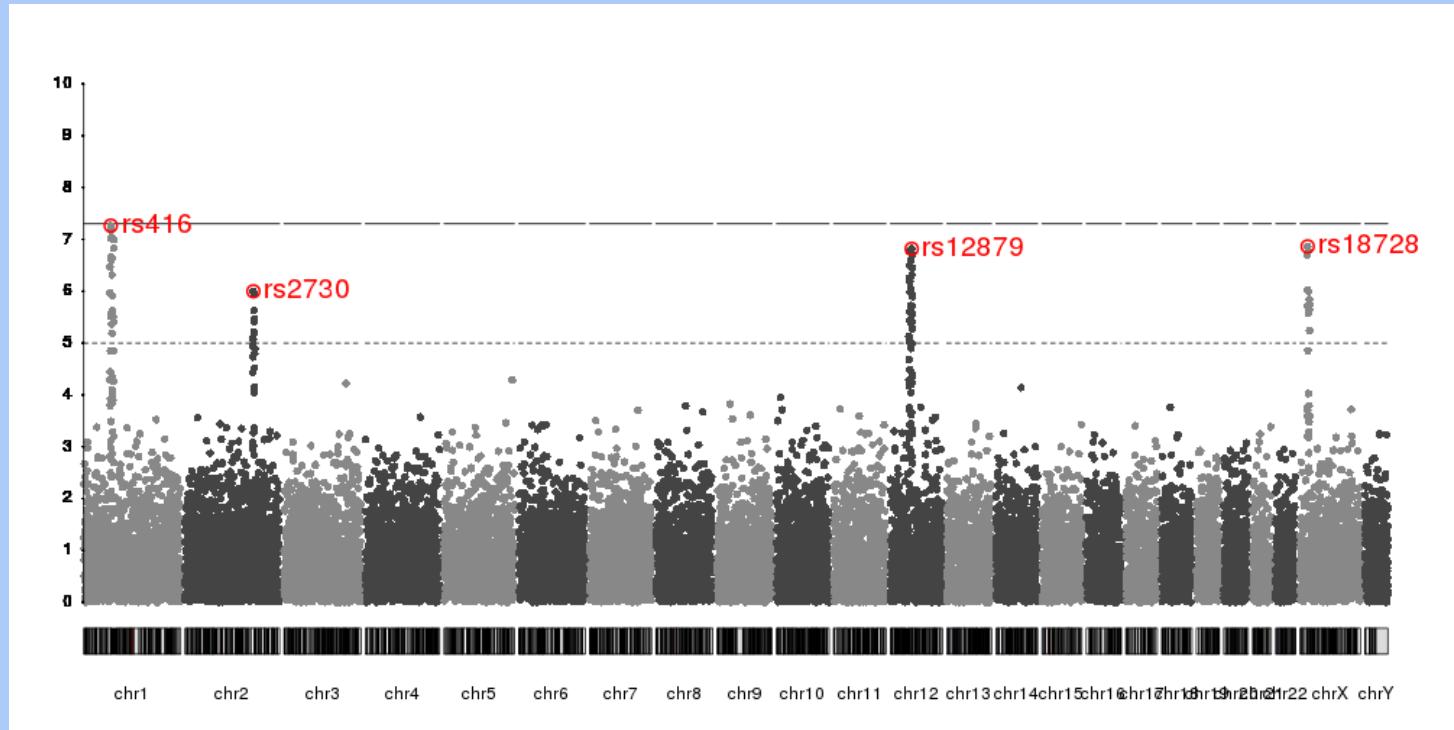


- If odds = a / b, then prob = a / (a + b) = odds / (1 + odds)

# Estimating the SNP effect

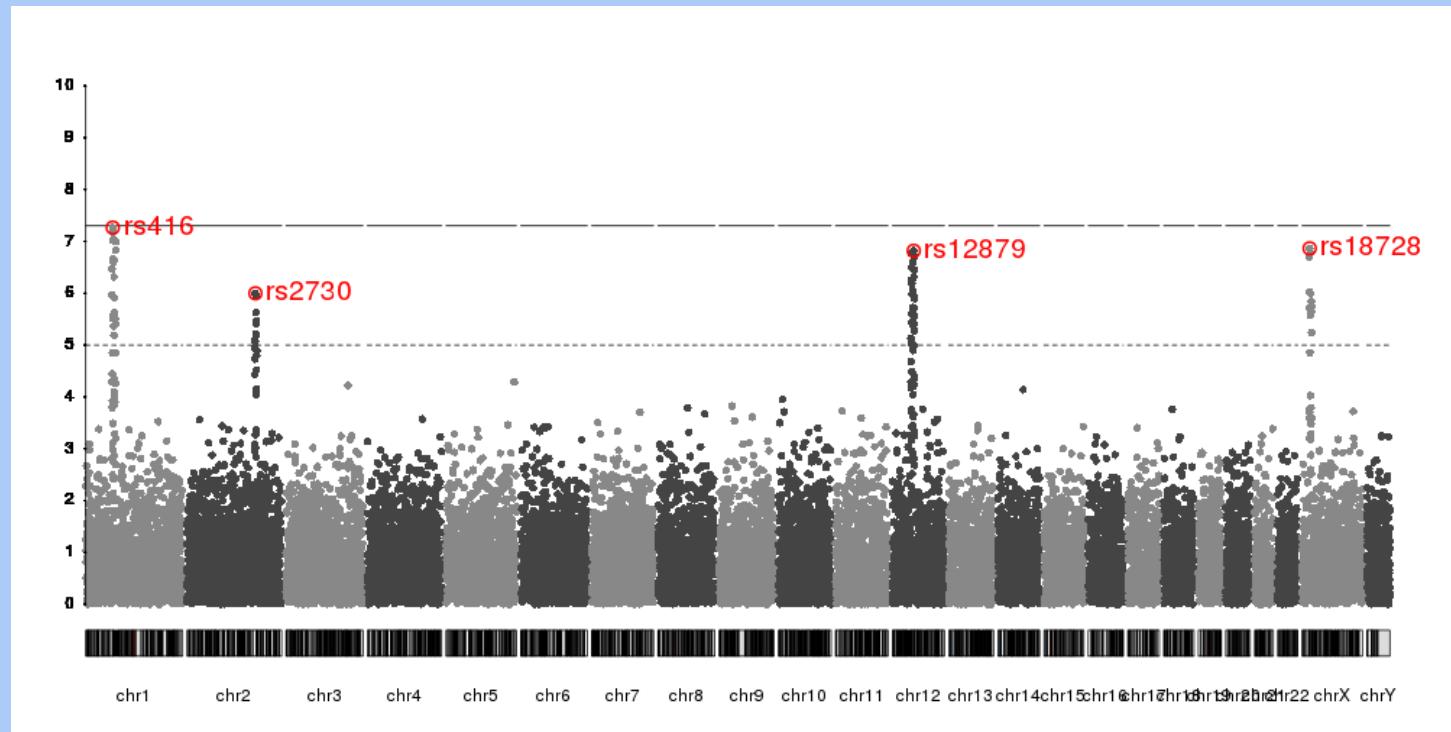
- We want to be able to **detect** the association of one SNP with disease by fitting the model  $Y = \beta_0 + \beta_1 X_1 + \dots$  and finding a slope  $\beta_1$  significantly different from 0

# Estimating the SNP effect



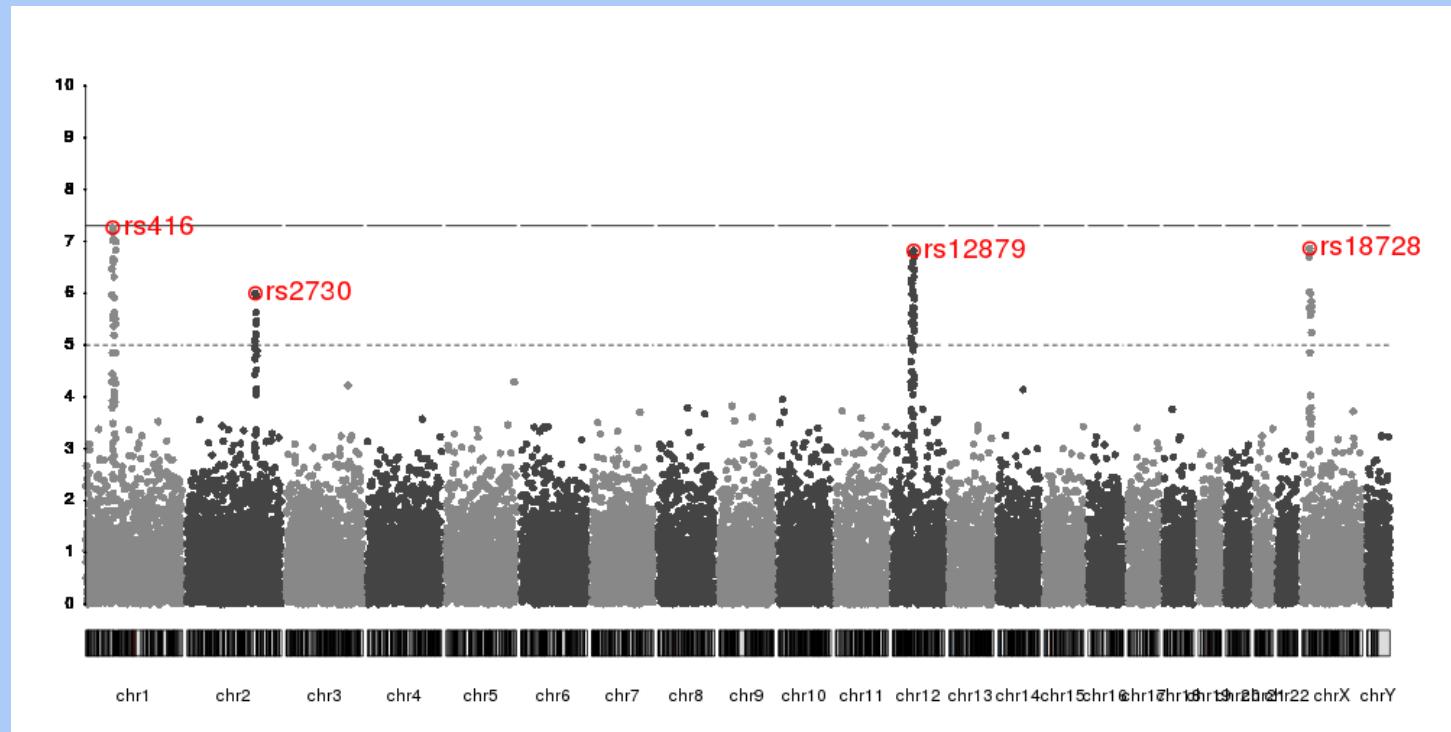
- A **Manhattan plot** gives the p-value of the log-OR estimate for each SNP

# Estimating the SNP effect



- Because there are more SNPs than subjects, we cannot fit all SNPs at once

# Estimating the SNP effect

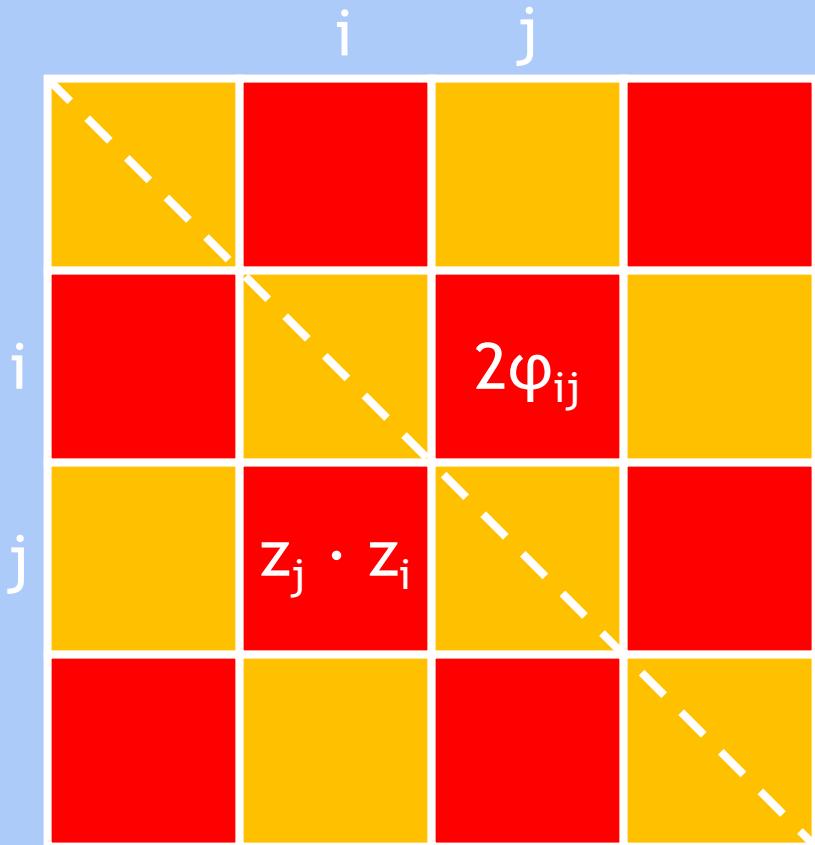


- But we can fit one SNP plus the “average” effect of all the remaining SNPs

# Linear mixed models

- The solution for the **best estimate** of the SNP effect  $\beta_1$  in the presence of all the remaining SNPs involves the GRM  $ZZ^T$  (from PC-Relate)

$$\mathbf{X}^T (\mathbf{I} + \mathbf{Z}\mathbf{Z}^T)^{-1} \hat{\beta} = \mathbf{X}^T (\mathbf{I} + \mathbf{Z}\mathbf{Z}^T)^{-1} \mathbf{Y}$$



# Linear mixed models

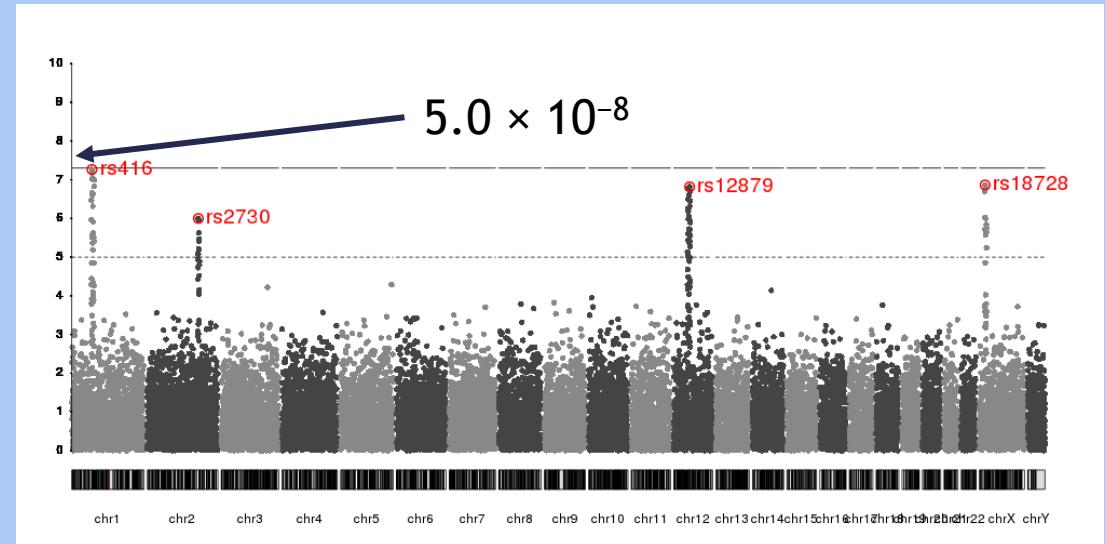
- Other covariates  $X$  commonly included in the model are age, sex, and the **first few genotype principal components** (from PC-AiR)

# Linear mixed models

- If the model including the SNP represents a significant improvement over the **null model** (the model without the SNP), we can reject the null hypothesis that the OR = 1

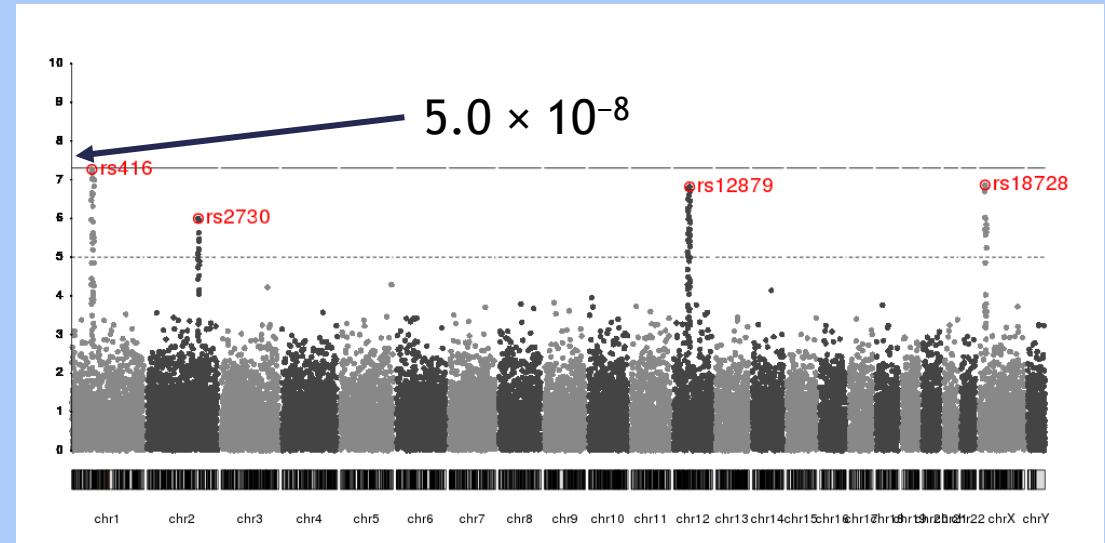
# Linear mixed models

- But because of **multiple-testing**, our p-value threshold is  $0.05 / 10^6$  (i.e., you perform the same test  $10^6$  times)



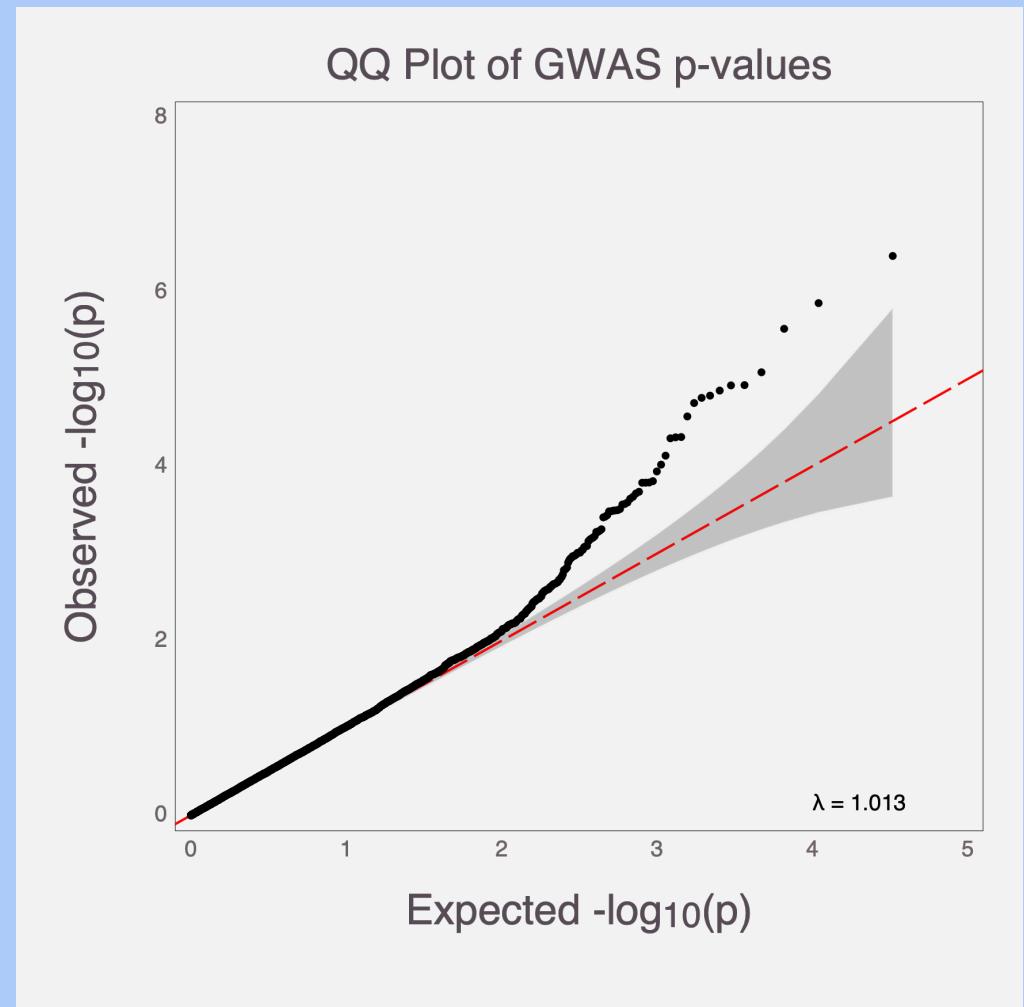
# Linear mixed models

- SNPs with  $p < 5.0 \times 10^{-8}$  are said to achieve genome-wide significance



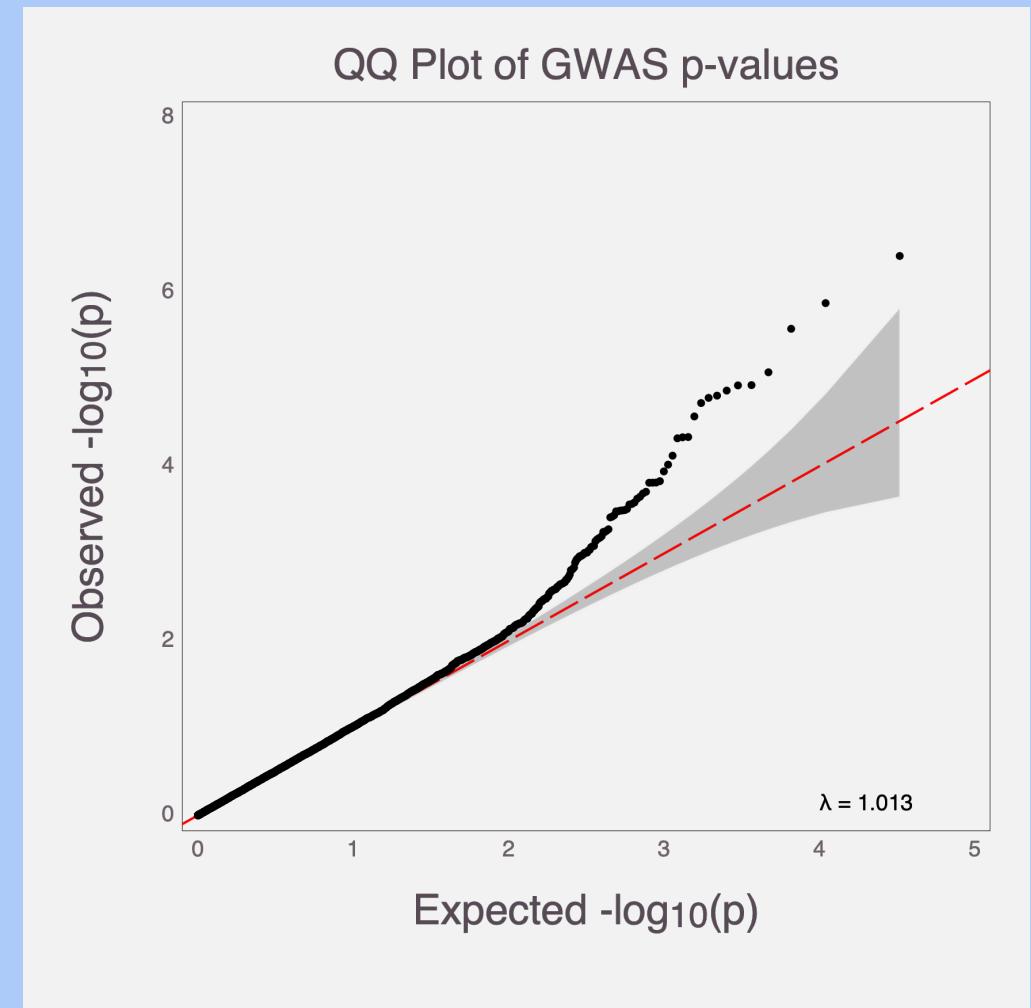
# QQ plots

- To assess if the distribution of SNP effects is significantly different from that expected by chance, we make a quantile or QQ plot



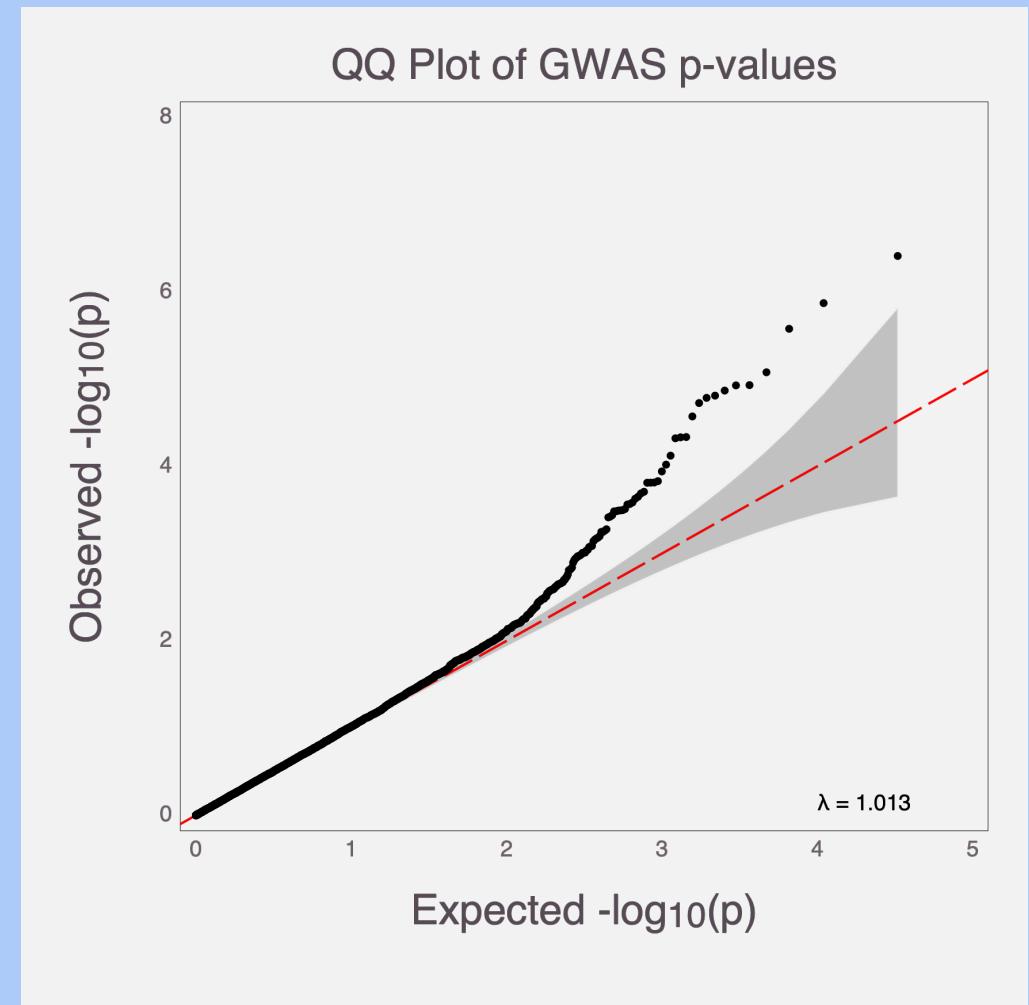
# QQ plots

- Put the **observed** p-value (negative log-10) in order from smallest to biggest



# QQ plots

- The **expected** p-values for the quantiles of  $m$  SNPs, are  $1/m, 2/m, \dots, 1$
- Take the negative log-10 and put in order from smallest to biggest



# QQ plots

- SNPs falling above the line of identity indicate an excess of quantiles ( $\beta$ 's) with small p-values

