# Concepts in complex diseases: from Fisher to GWAS

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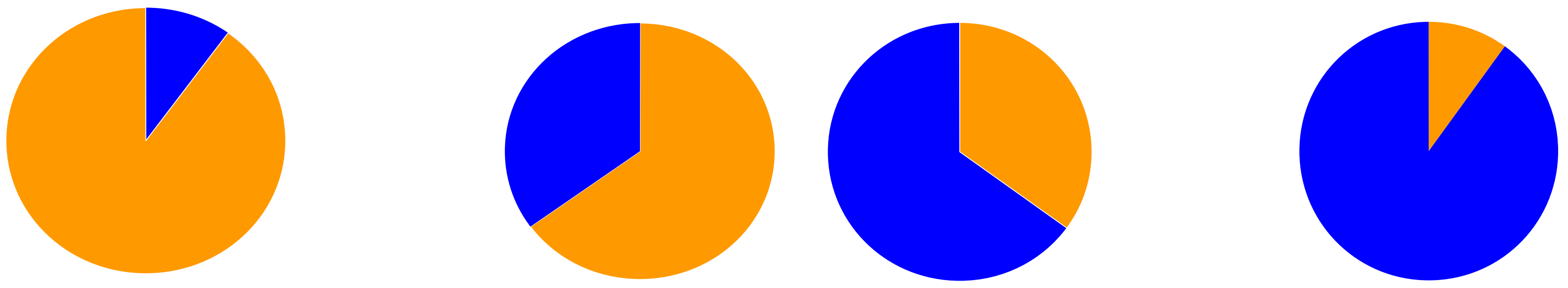
# Approaches to study complex diseases

- Introduction: polygenic theory of Fisher
- Linkage disequilibrium
- How to identify genes for complex diseases?
- What has been accomplished today?
- Pitfalls of genetic association studies
  - Multiple testing
  - Missing heritability

# Genes and Disease



## Monogenic Diseases

- Huntington Disease
- Cystic fibrosis
- Fragile X syndrome

## Complex Diseases

- Alzheimer disease
- Cardiovascular Disease
- Autism

## Environmental Diseases

- Pathogens
- Poisoning

🟦 Environment 🟧 Genes

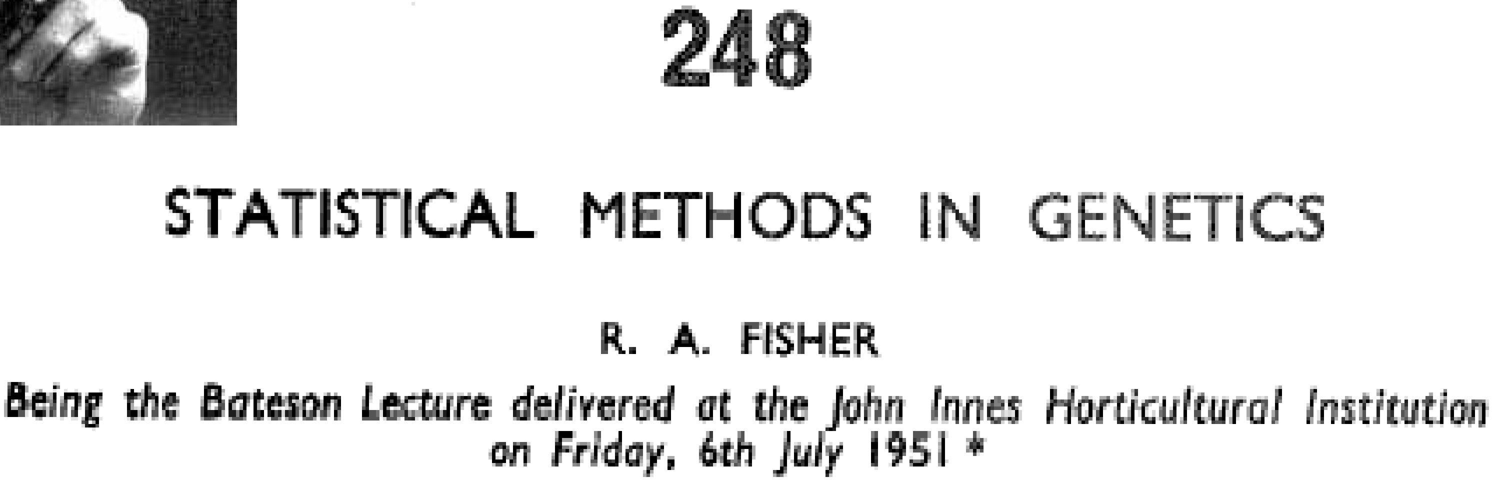
Some differences …

| Mendelian disorder | Complex disorder |
|---|---|
| • Mutation in 1 gene is sufficient to cause the disorder | • Mutation in a gene confers an increased risk, but is not sufficient to cause the disorder |
| • Recognizable inheritance patterns | • No clear inheritance pattern |
| • One gene per family | • Involves many genes or genes and environment |
| • Less common diseases | • Many are common diseases |

# Complex traits: polygenic theory

Sir Ronald Aylmer Fisher (1890-1962)

- Created the foundations for modern statistical science
- Reconciled the discontinuous nature of Mendelian inheritance with continuous variation



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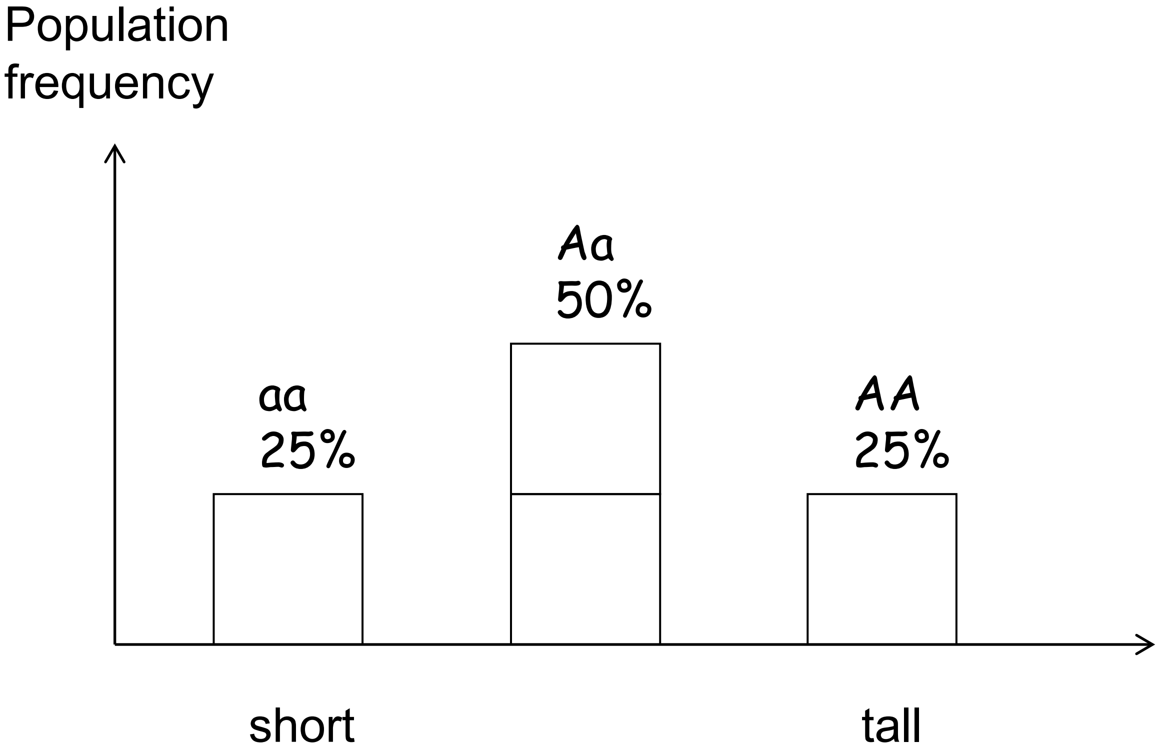
STATISTICAL METHODS IN GENETICS

R. A. FISHER

*Being the Bateson Lecture delivered at the John Innes Horticultural Institution on Friday, 6th July 1951 **

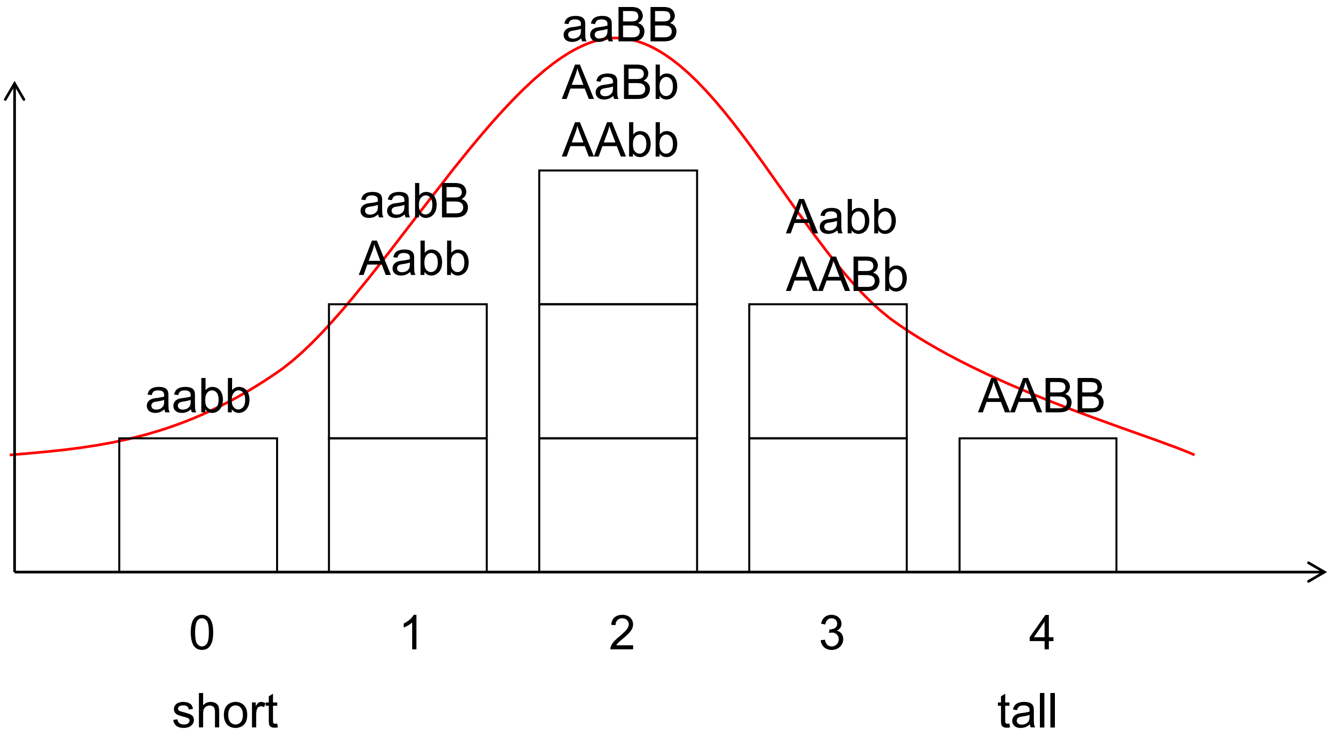
# Complex traits: polygenic theory

Example: body length
Suppose simple monogenic trait with **one gene A**, two alleles



# Suppose simple genetic trait with **two genes A and B**, 4 alleles

| | | | | |
|---|---|---|---|---|
| aabb | aabB Aabb | aaBB AaBb AAbb | AaBB AABb | AABB |
| 0 | 1 | 2 | 3 | 4 |

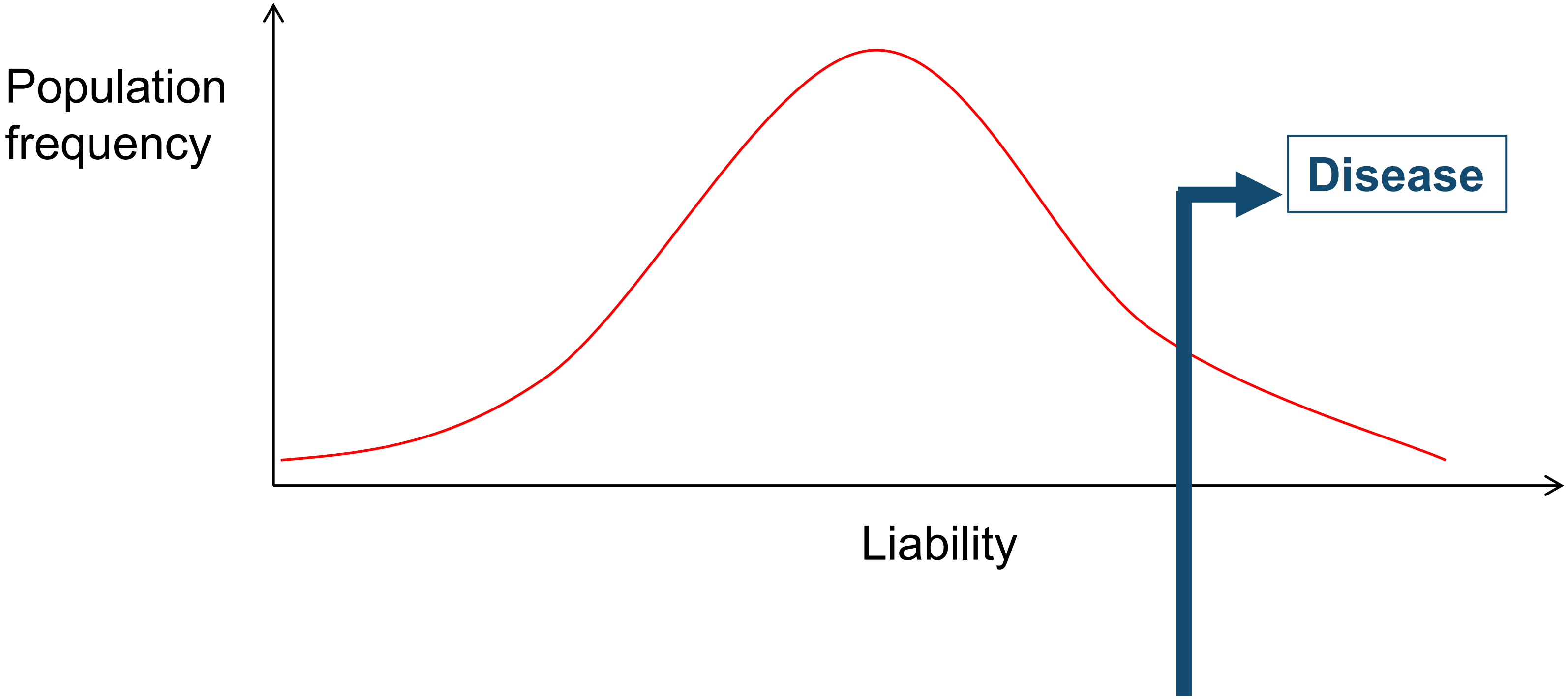


# Binary traits (health-disease)

Some traits are binary, not continuous

e.g. Disease or health

Liability distribution, threshold model

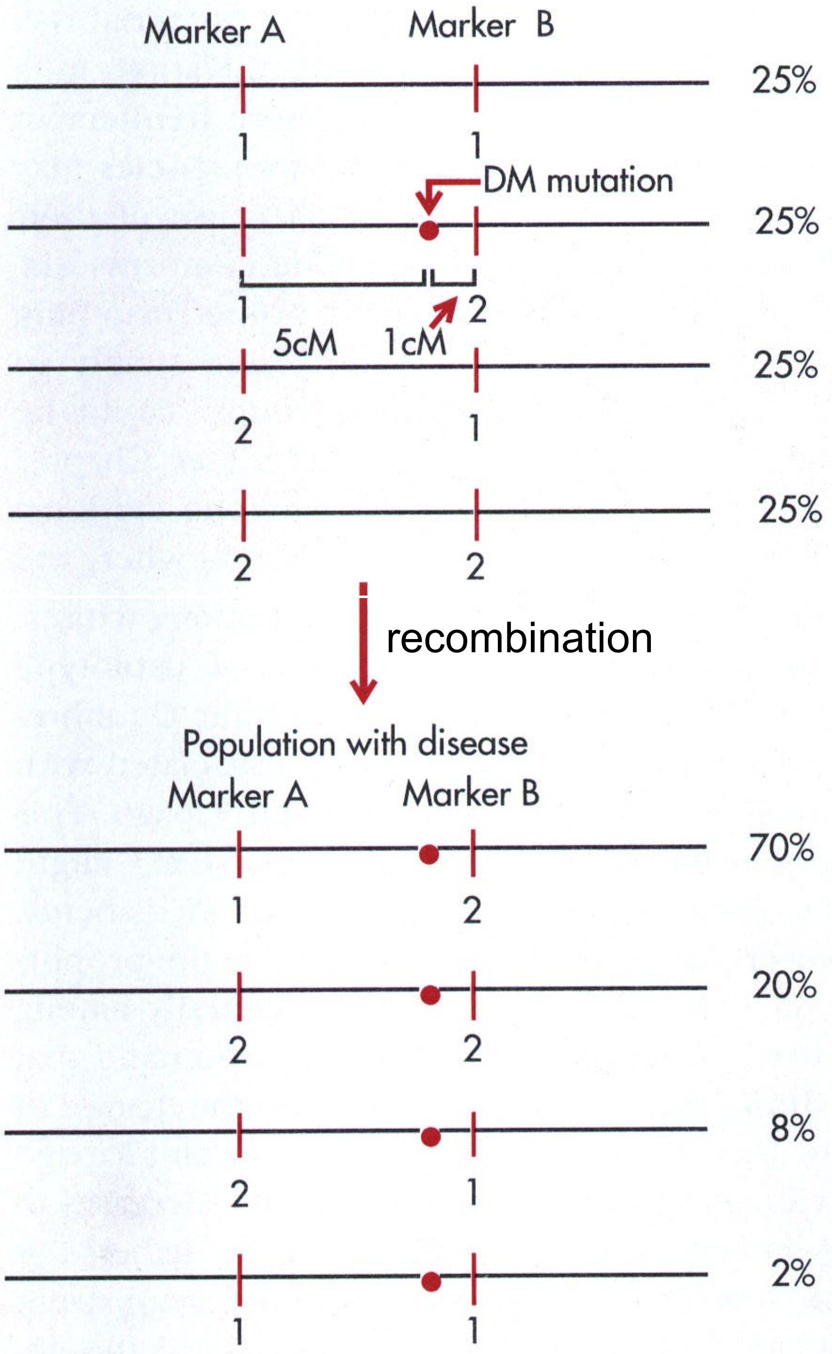


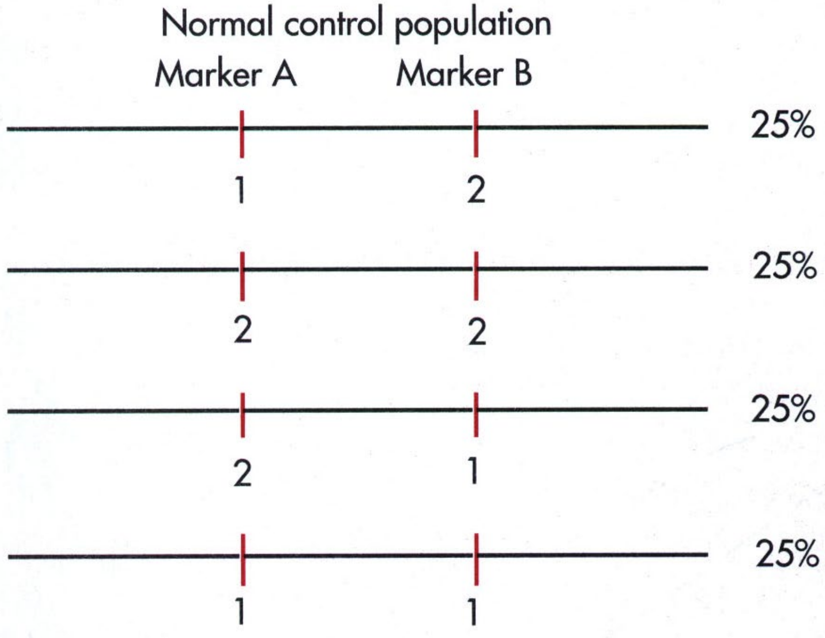
# Linkage disequilibrium (LD)

- Linkage Disequilibrium is the non-random association of alleles at two or more loci
- Some haplotypes occur more or less frequently than would be expected on the basis of their allele frequencies
- Can occur between a disease mutation and markers
  - Monogenic diseases (e.g. myotonic dystrophy, cystic fibrosis)
  - Complex diseases
  - Due to common ancestor
- Can occur between DNA variants

# LD between a mutation and markers

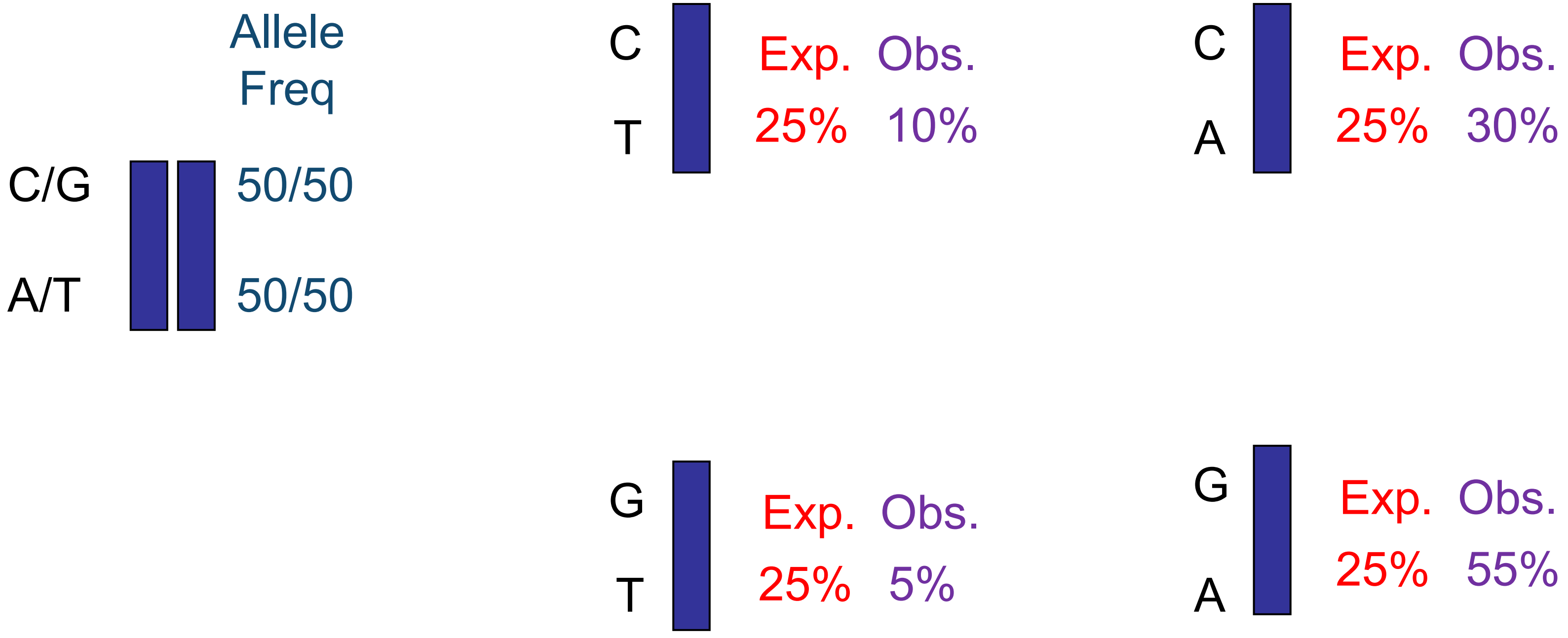
- Without recombination 100% of the mutations is on the original haplotype
- Mutations on another haplotype originate by recombination





# LD between SNPs

2 SNPs closely together: expected and observed haplotype frequencies



Allele Freq

C/G 50/50

A/T 50/50

C T — Exp. 25% Obs. 10%

C A — Exp. 25% Obs. 30%

G T — Exp. 25% Obs. 5%

G A — Exp. 25% Obs. 55%

4 haplotypes

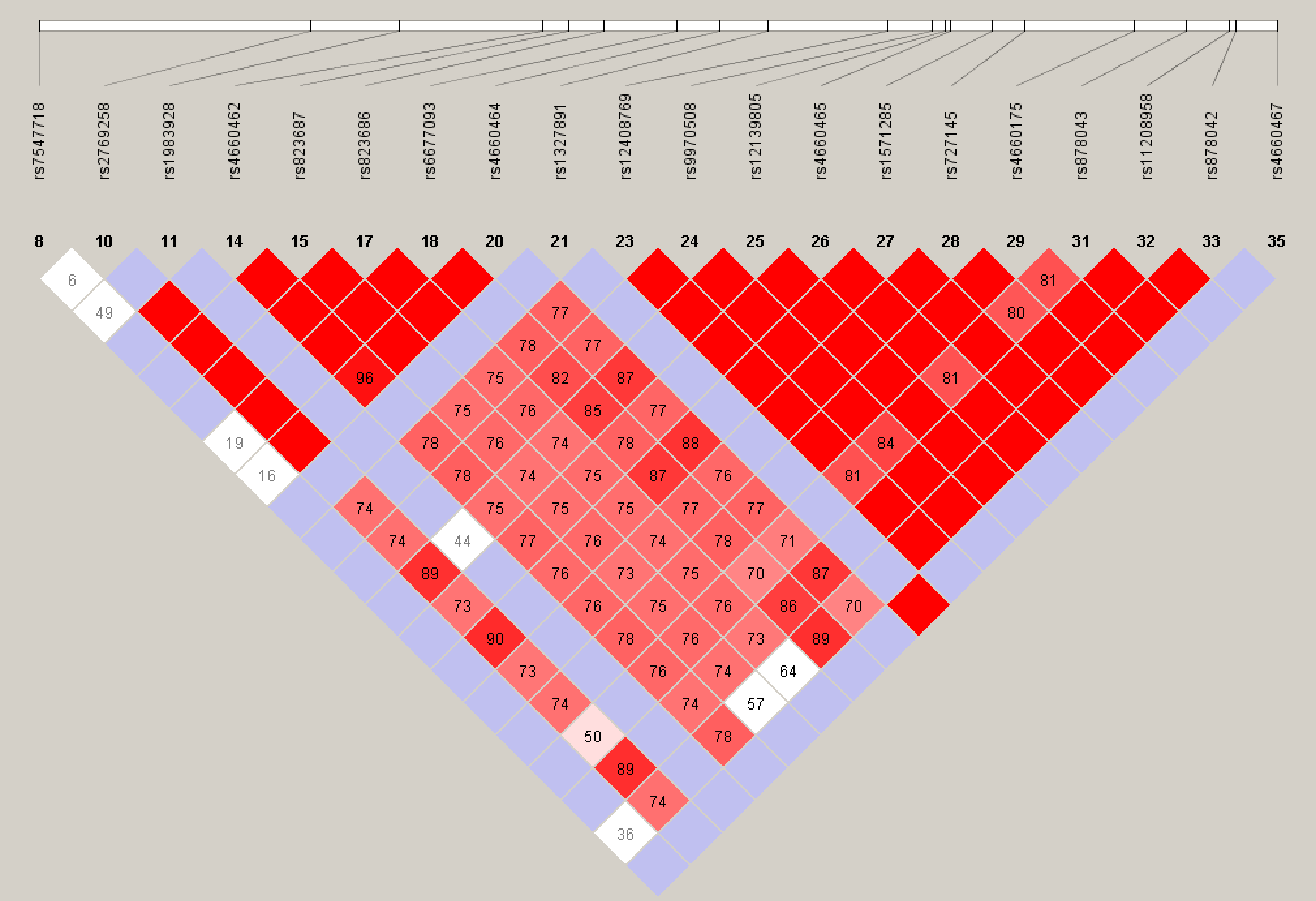
# LD decay

- A new SNP allele that arises by mutation is in LD with all surrounding alleles of the haplotype on which it arose
- LD breaks down by recombination
- Remaining LD is due to lack of historic recombination between adjacent markers
  - On average, pairwise LD decays with distance between SNPs
  - Over short distances, this decay is not a smooth function, rather stepwise

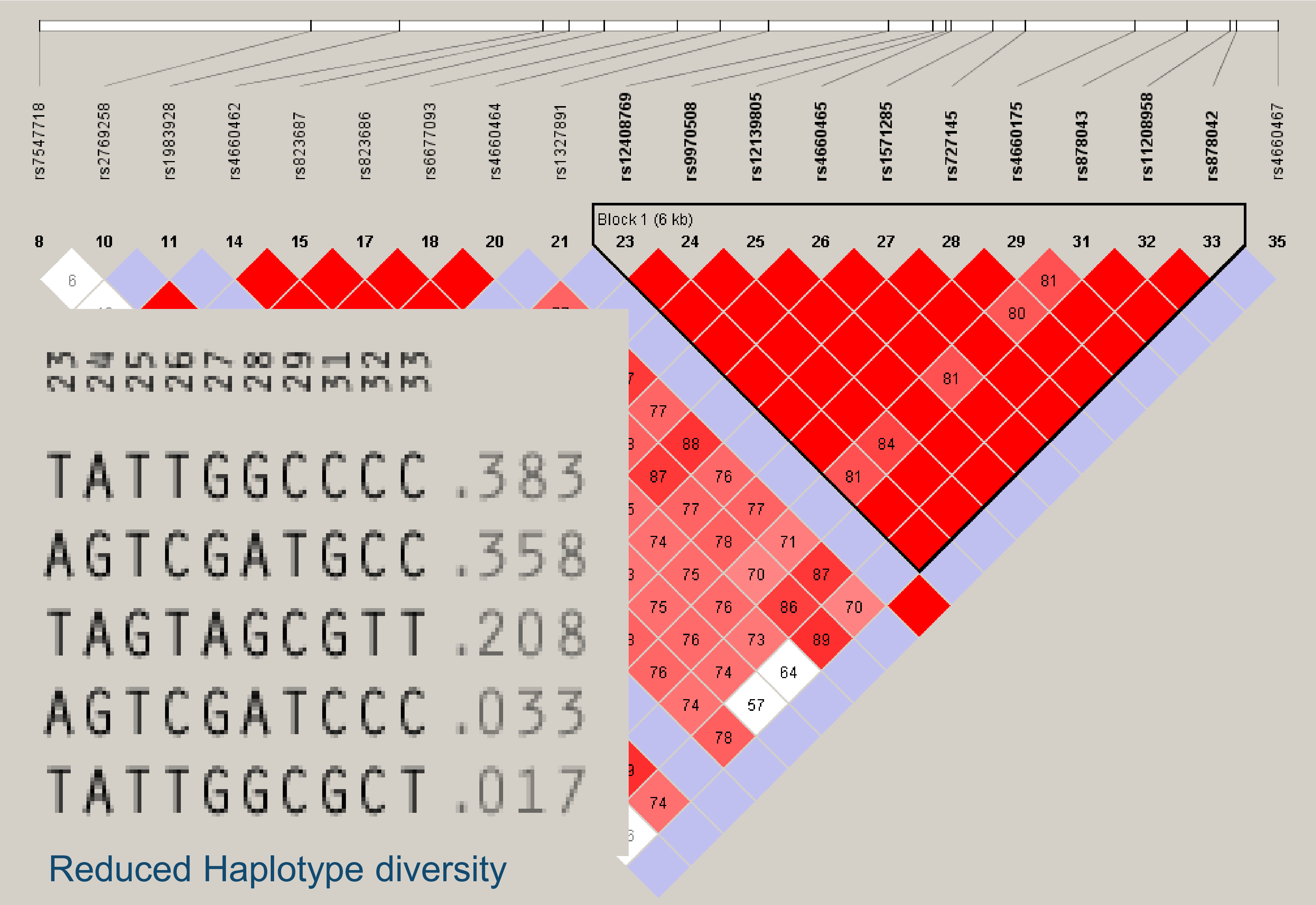
# LD blocks

- Long stretch of markers in LD followed by recombination hotspot
- LD block:
  - region of high LD between adjacent SNPs
  - region of limited haplotype diversity
- Blocks are found over entire genome, but boundaries not always clear

# LD structure in Haploview



# LD structure in Haploview

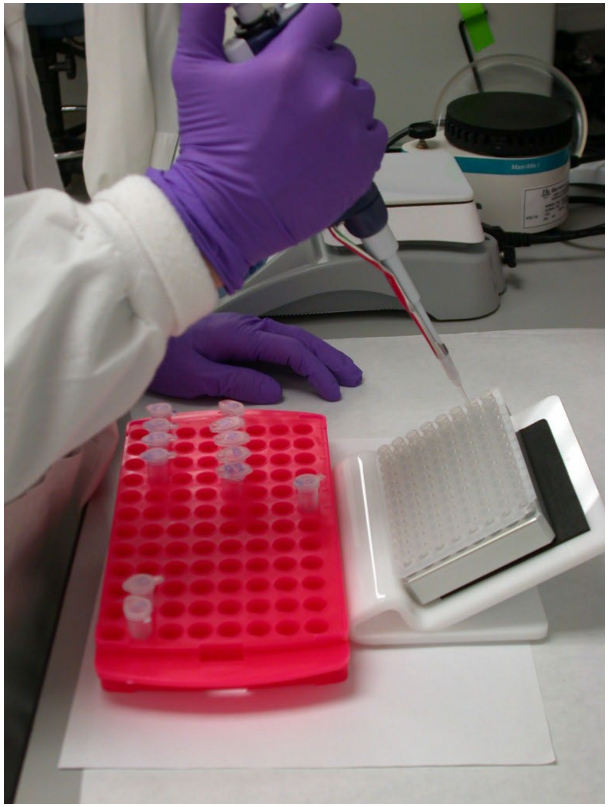


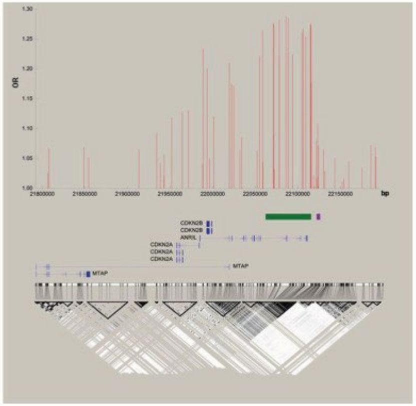
```
2 2 2 2 2 2 2 3 3 3
3 4 5 6 7 8 9 1 2 3

TATTGGCCCC .383
AGTCGATGCC .358
TAGTAGCGTT .208
AGTCGATCCC .033
TATTGGCGCT .017
```

Reduced Haplotype diversity

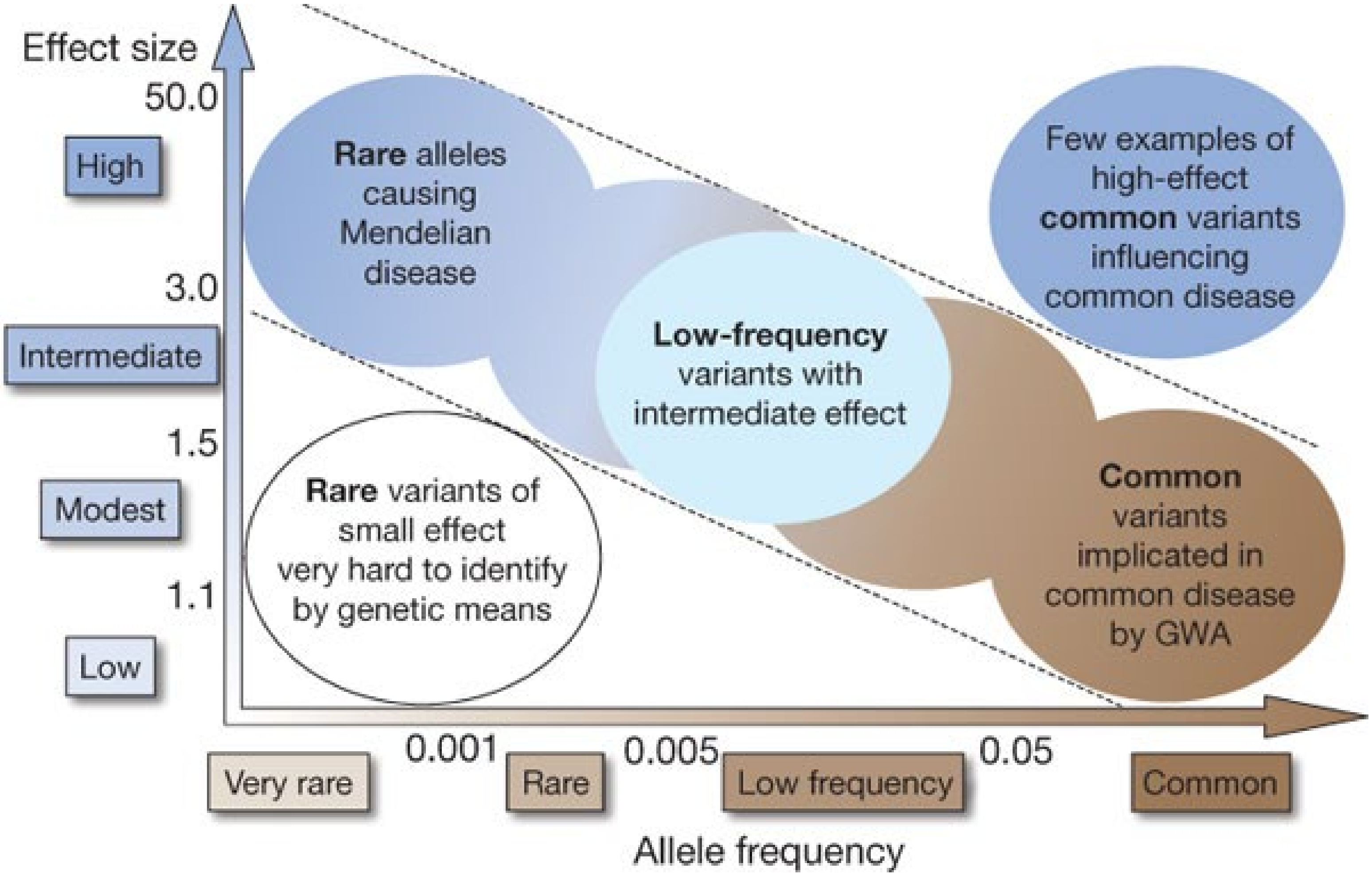
# How to identify genes for complex phenotypes?





# Feasibility of identifying disease genes

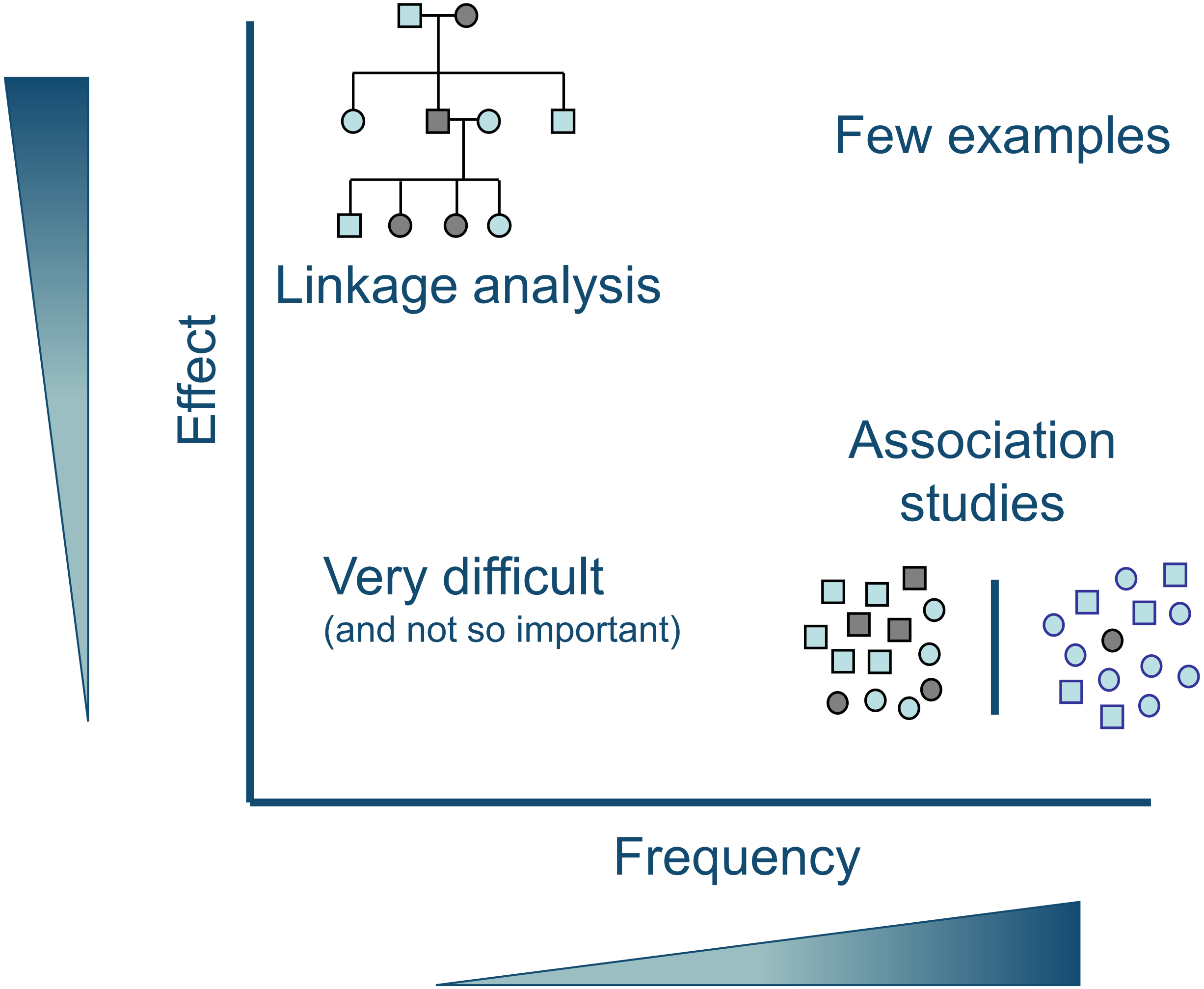
odds ratio



Effect size
50.0
High
3.0
Intermediate
1.5
Modest
1.1
Low
Rare alleles causing Mendelian disease
Few examples of high-effect common variants influencing common disease
Low-frequency variants with intermediate effect
Rare variants of small effect very hard to identify by genetic means
Common variants implicated in common disease by GWA
Very rare
0.001
Rare
0.005
Low frequency
0.05
Common
Allele frequency

Manolio *et al.* *Nature* **461**, 747-753 (2009)

# Popular methods for disease gene identification



Effect

Linkage analysis

Few examples

Very difficult
(and not so important)

Association studies

Frequency

Protective

… ATGCAATGAC …

… ATGCAATGAC …

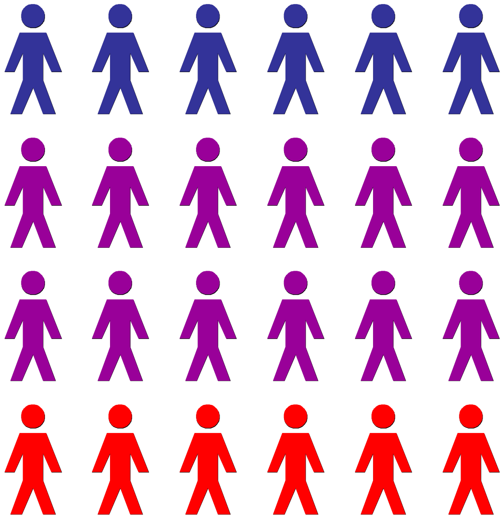
… ATGCAATGAC …

… ATGCATTGAC …

… ATGCATTGAC …

… ATGCATTGAC …

Risk

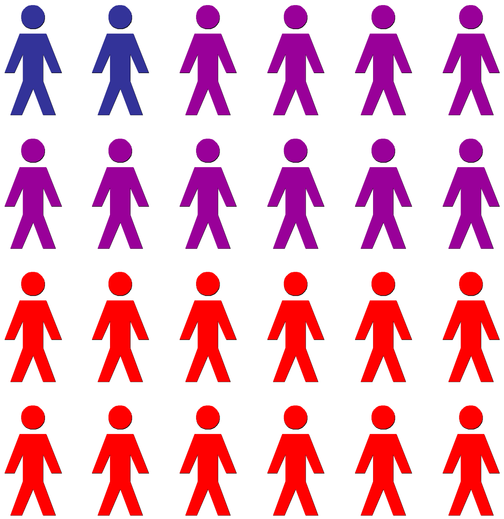


General population

SNP: AA

SNP: TT

SNP: AT



Patients

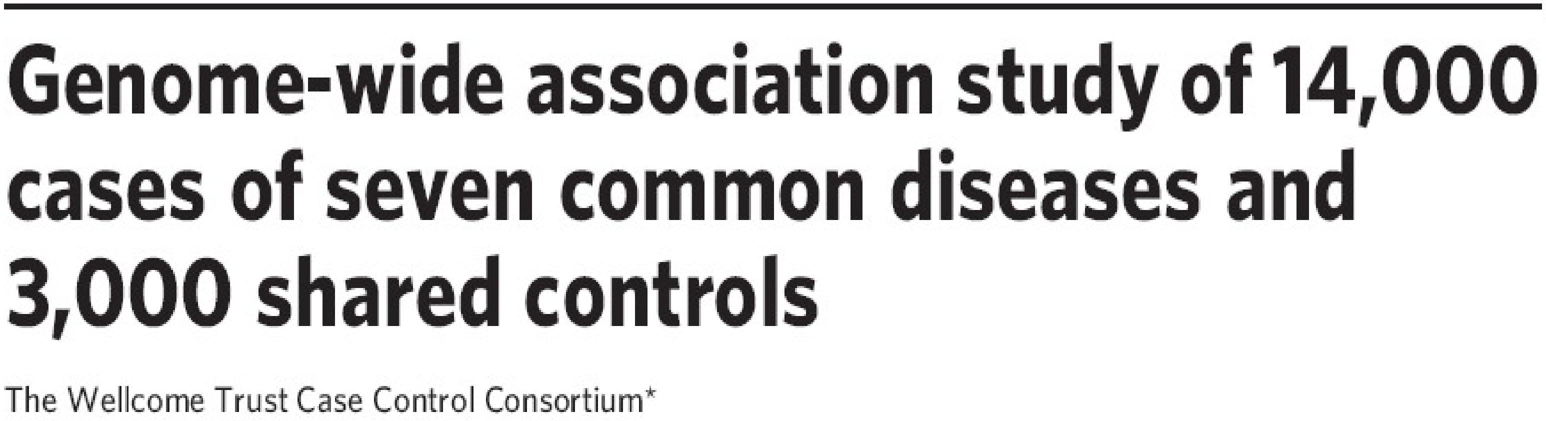
# Genome-wide Association Studies (GWAS)

- Total genome: 10 million SNPs
  - HapMap: 4 million SNPs in CEU
- Due to LD: no need to type all SNPs
  - tagSNPs on chip give info on non-typed SNPs: imputation of non-typed SNPs is possible
  - 500,000 to $10^6$ SNPs on a chip
  - Illumina 550 K using tagSNPs : 89% coverage ($r^2 > 0.8$)

# LD : strength or weakness ?

- Pro: Can pick up association through surrounding markers in LD

- Con: If you find an associated SNP, you can’t be sure it’s the causative one



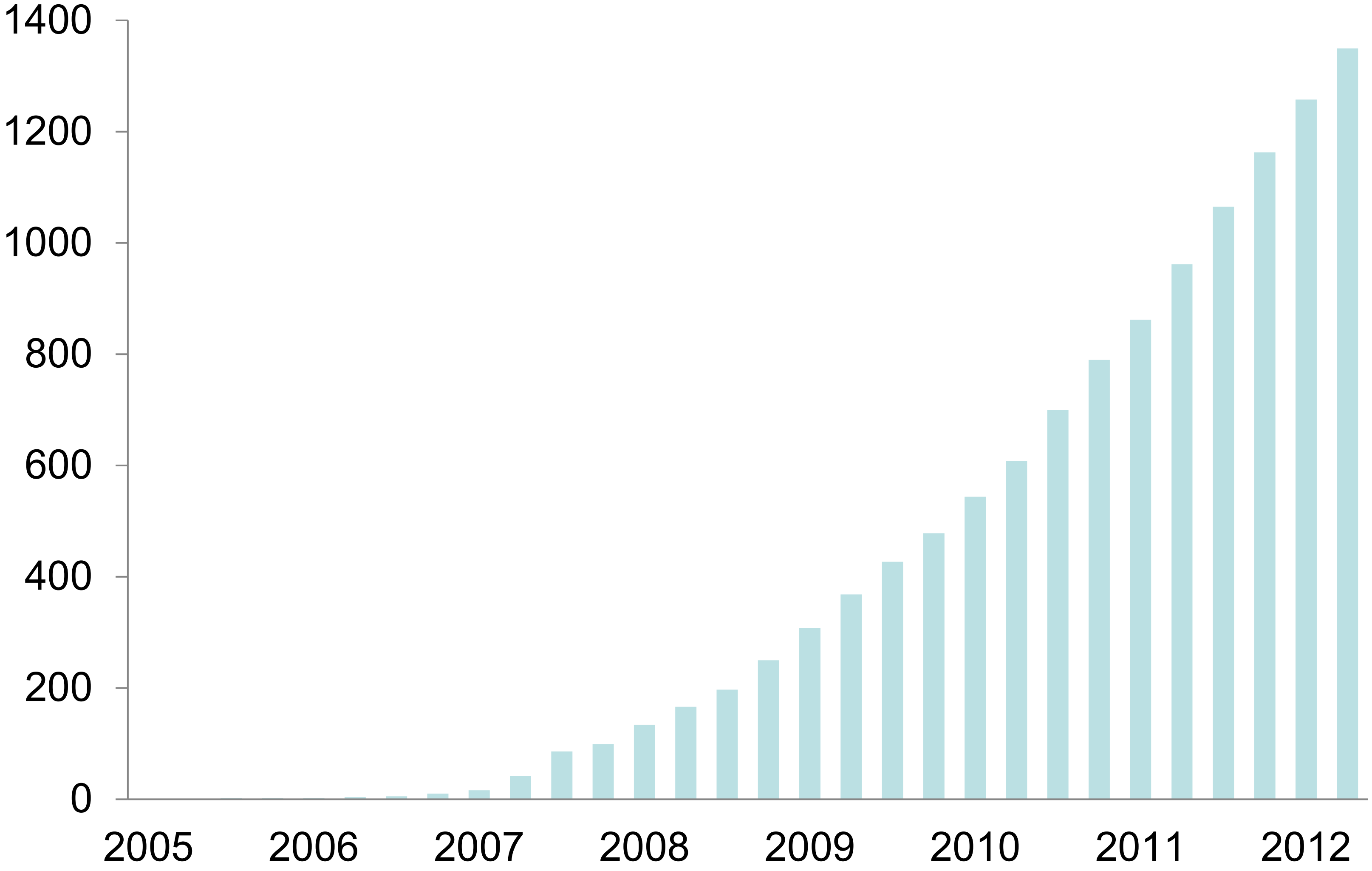
# Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls

The Wellcome Trust Case Control Consortium*

Nature, June 2007

- 2000 patients for 7 diseases, 3000 controls
- 500,000 SNPs analysed
- 24 clear signals
- Small effects
- Replication = gold standard

# Published GWA Reports, 2005 – 6/2012

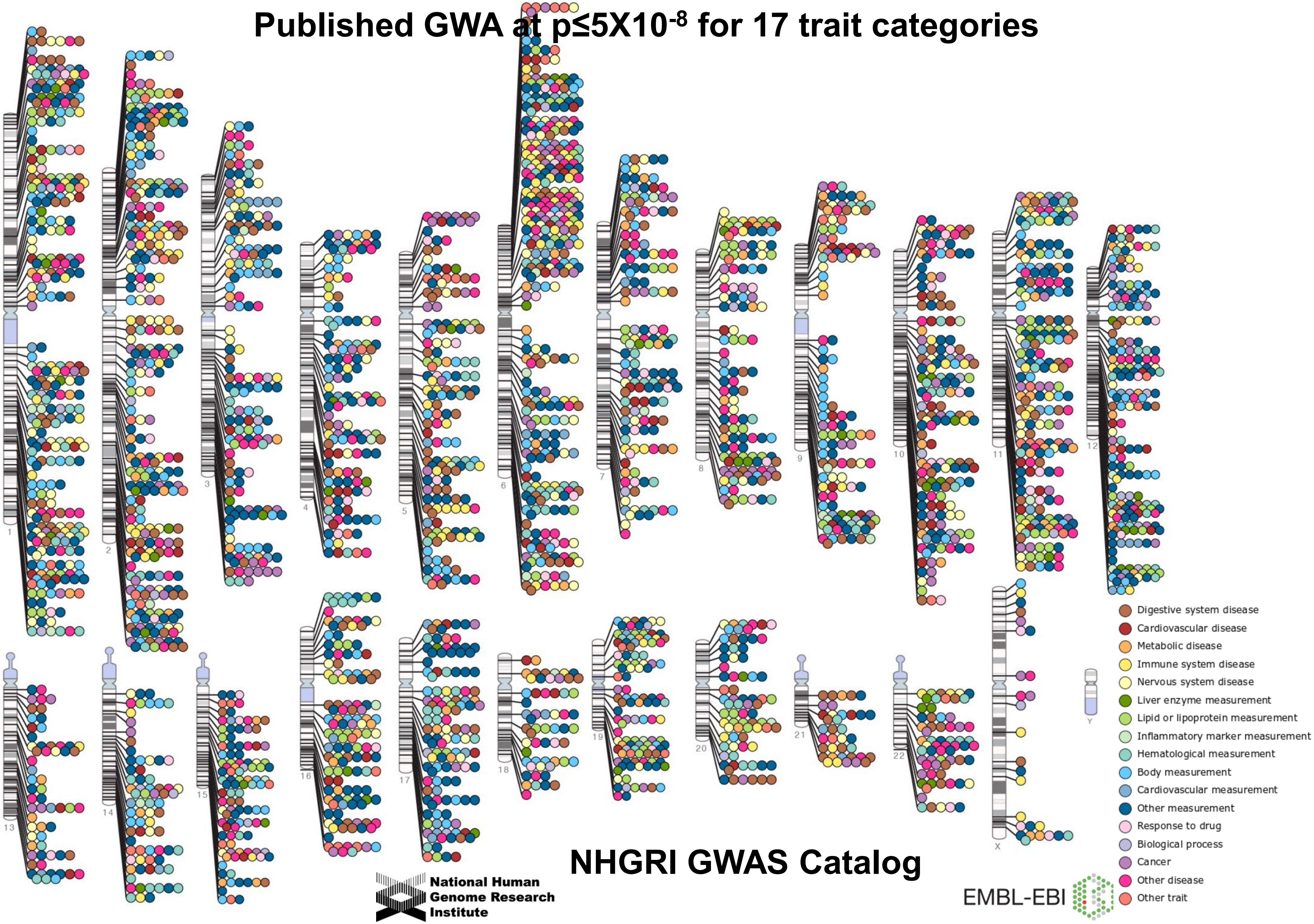


**NHGRI GWA Catalog**
**www.genome.gov/GWAStudies**

**Through 6/30/12 postings**

# Published Genome-Wide Associations through 12/2012

# Published GWA at $p \leq 5X10^{-8}$ for 17 trait categories



## NHGRI GWAS Catalog

National Human
Genome Research
Institute

EMBL-EBI

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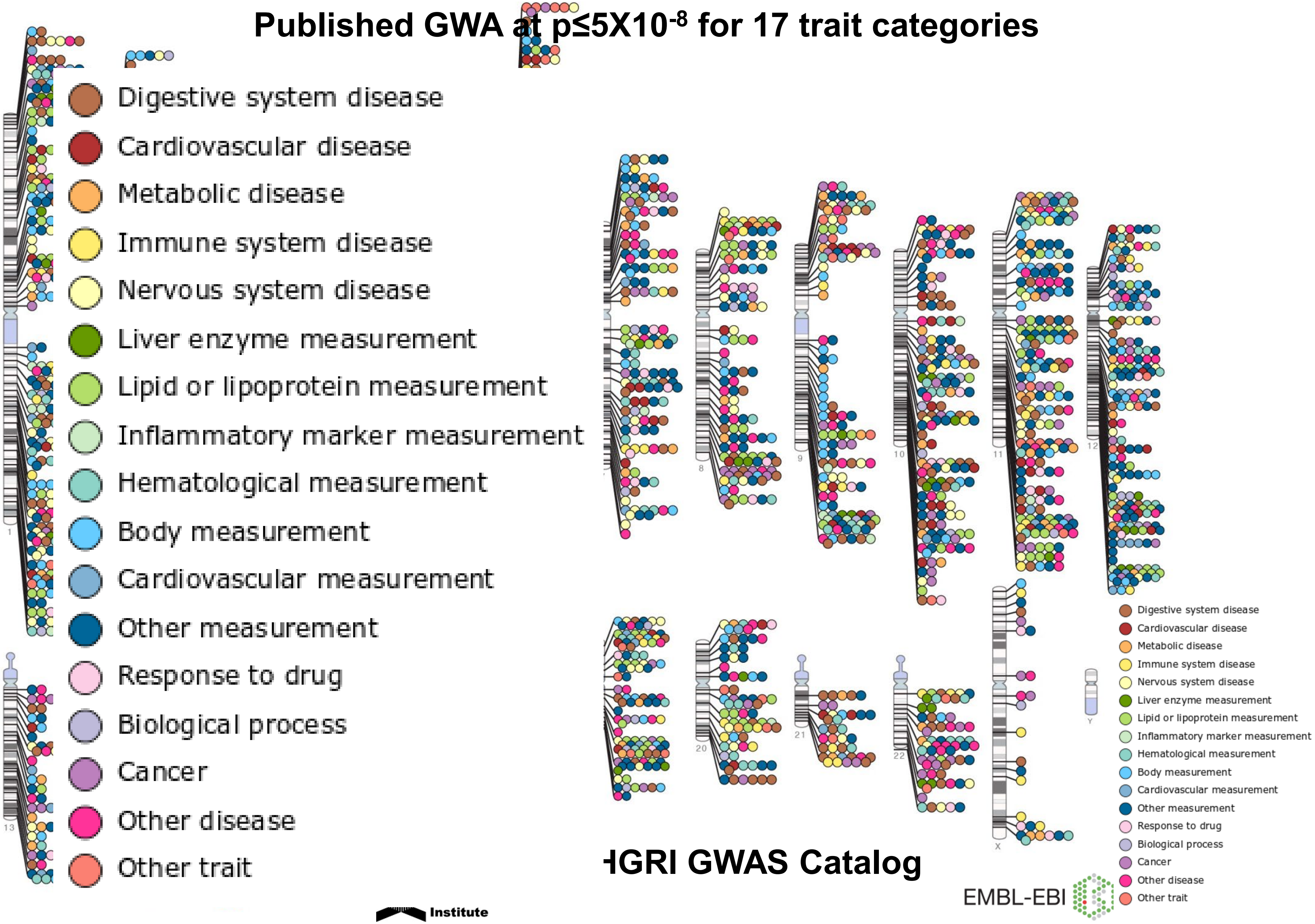


- ● Digestive system disease
- ● Cardiovascular disease
- ● Metabolic disease
- ● Immune system disease
- ● Nervous system disease
- ● Liver enzyme measurement
- ● Lipid or lipoprotein measurement
- ● Inflammatory marker measurement
- ● Hematological measurement
- ● Body measurement
- ● Cardiovascular measurement
- ● Other measurement
- ● Response to drug
- ● Biological process
- ● Cancer
- ● Other disease
- ● Other trait

HGRI GWAS Catalog

EMBL-EBI

Institute

# Body length: 90 years after Fisher

*Nature Genetics* **40**, 575 - 583 (2008)

**Genome-wide association analysis identifies 20 loci that influence adult height**

Michael N Weedon, Hana Lango, Cecilia M Lindgren, Chris Wallace, David M Evans, Massimo Mangino, Rachel M Freathy, John R B Perry, Suzanne Stevens, Alistair S Hall, Nilesh J Samani, Beverly Shields, Inga Prokopenko, Martin Farrall, Anna Dominiczak, Diabetes Genetics Initiative, The Wellcome Trust Case Control Consortium, Toby Johnson, Sven Bergmann, Jacques S Beckmann, Peter Vollenweider, Dawn M Waterworth, Vincent Mooser, Colin N A Palmer, Andrew D Morris, Willem H Ouwehand, Cambridge GEM Consortium, Mark Caulfield, Patricia B Munroe, Andrew T Hattersley, Mark I McCarthy & Timothy M Frayling

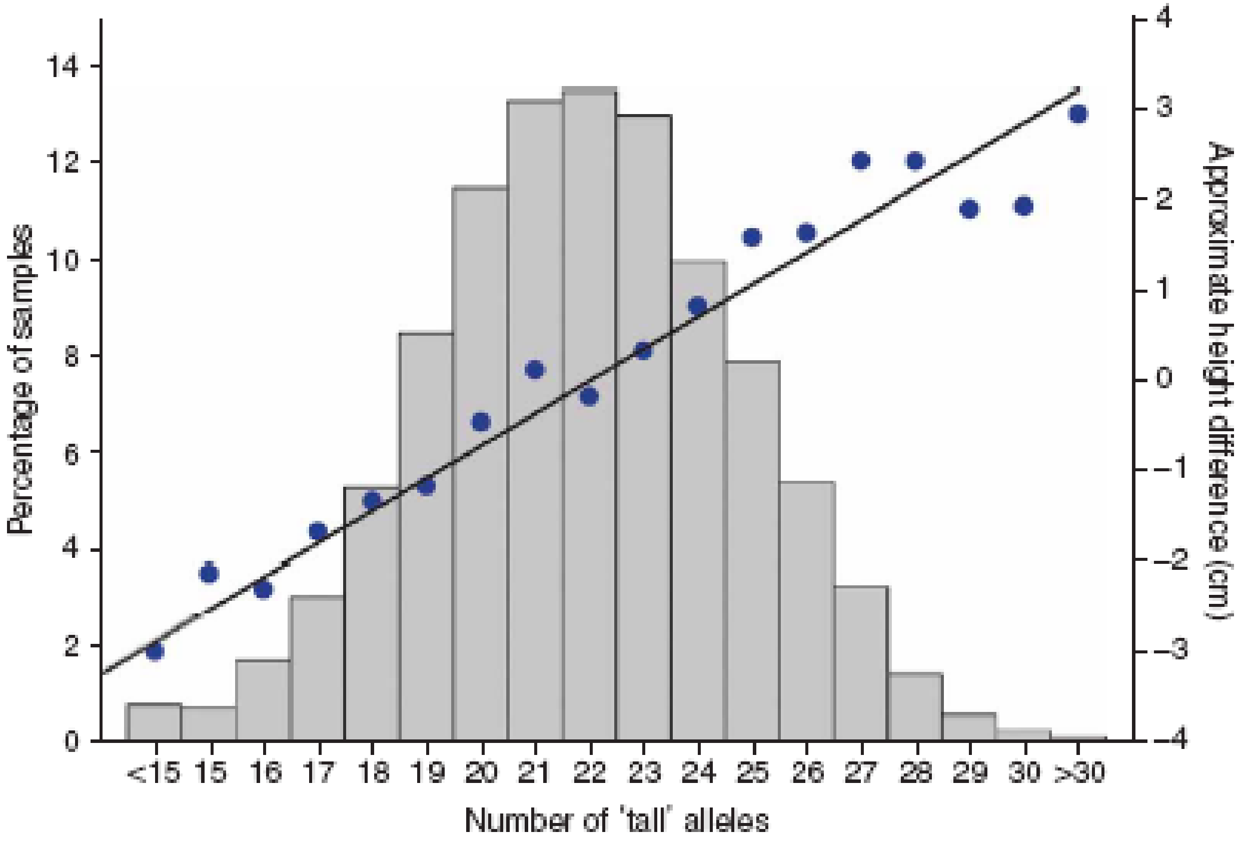
# Body length: 90 years after Fisher

- Heritability close to 1
- Weedon et al. (2008): Association tested in GWAS on ~34,000 individuals
- Influenced by 20 genes
  - Each variant has ‘tall’ and ‘small’ allele
  - Body length ~ number of tall alleles
  - 6 cm difference between 15 and 30 tall alleles

# Body length

15 tall alleles

30 tall alleles



# More recent results on body length

- Nat Genet. 2014, 46:1173-86.
- 253,288 individuals
- 697 variants at genome-wide significance
  - together explained one-fifth of the heritability for adult height.
- All common variants together captured 60% of the heritability
- Enriched for genes, pathways and tissue types known to be involved in growth
- Several genes and pathways not previously connected with human skeletal growth

# Pitfalls of genetic association studies

- Multiple testing
- Missing heritability

# Multiple testing

- When is an association “proven”?
- Classical threshold of $p < 0.05$ ?
- 5% of the test are expected to be significant ($p<0.05$) just by chance
  - Testing 100 SNPs: expect 5 p-values $< 0.05$ by chance
  - Testing 500K SNPs: expect 25,000 p-values $< 0.05$ by chance
- Multiple testing leads to increased type I error ($\alpha$-error, false positive)

# Solutions for multiple testing problem

- Adjusting significance level
  - Declare significant if $p < 0.05/\#$ tests (Bonferroni correction)
  - Some think it is too strict for GWAS due to dependence of tests (LD)
  - Consensus GWAS significance threshold of $5 \times 10^{-8}$

    (Similar to LOD score genome wide threshold of 3.3)
- Replicate significant findings in independent population

# 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.

# Missing heritability

| Disease | Number of loci | Heritability explained |
|---|---|---|
| Age-related macular degeneration | 5 | 50% |
| Crohn's disease | 32 | 20% |
| Systemic lupus erythematosus | 6 | 15% |
| Type 2 diabetes | 18 | 6% |
| HDL cholesterol | 7 | 5.2% |
| Height | 40 | 5% |
| Early onset myocardial infarction | 9 | 2.8% |
| Fasting glucose | 4 | 1.5% |

*Manolio et al, Nature* **461**, 747-753, 2009

# Missing heritability

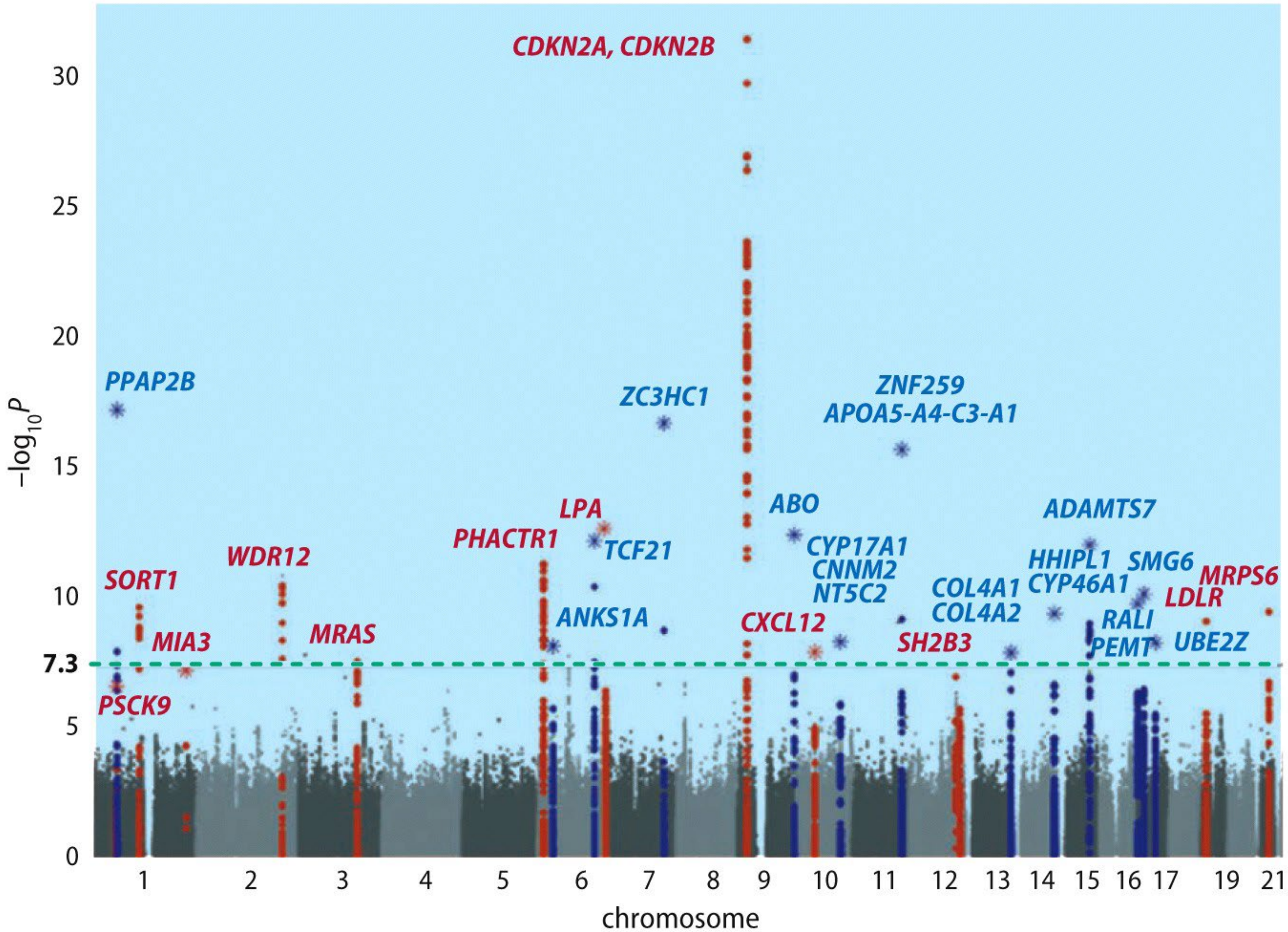
Possible origin:

- Variants (of smaller effect) yet to be found
- Rare variants
- Structural variants poorly captured by existing microarrays
- Gene–gene interactions
- Inadequate accounting for shared environment among relatives

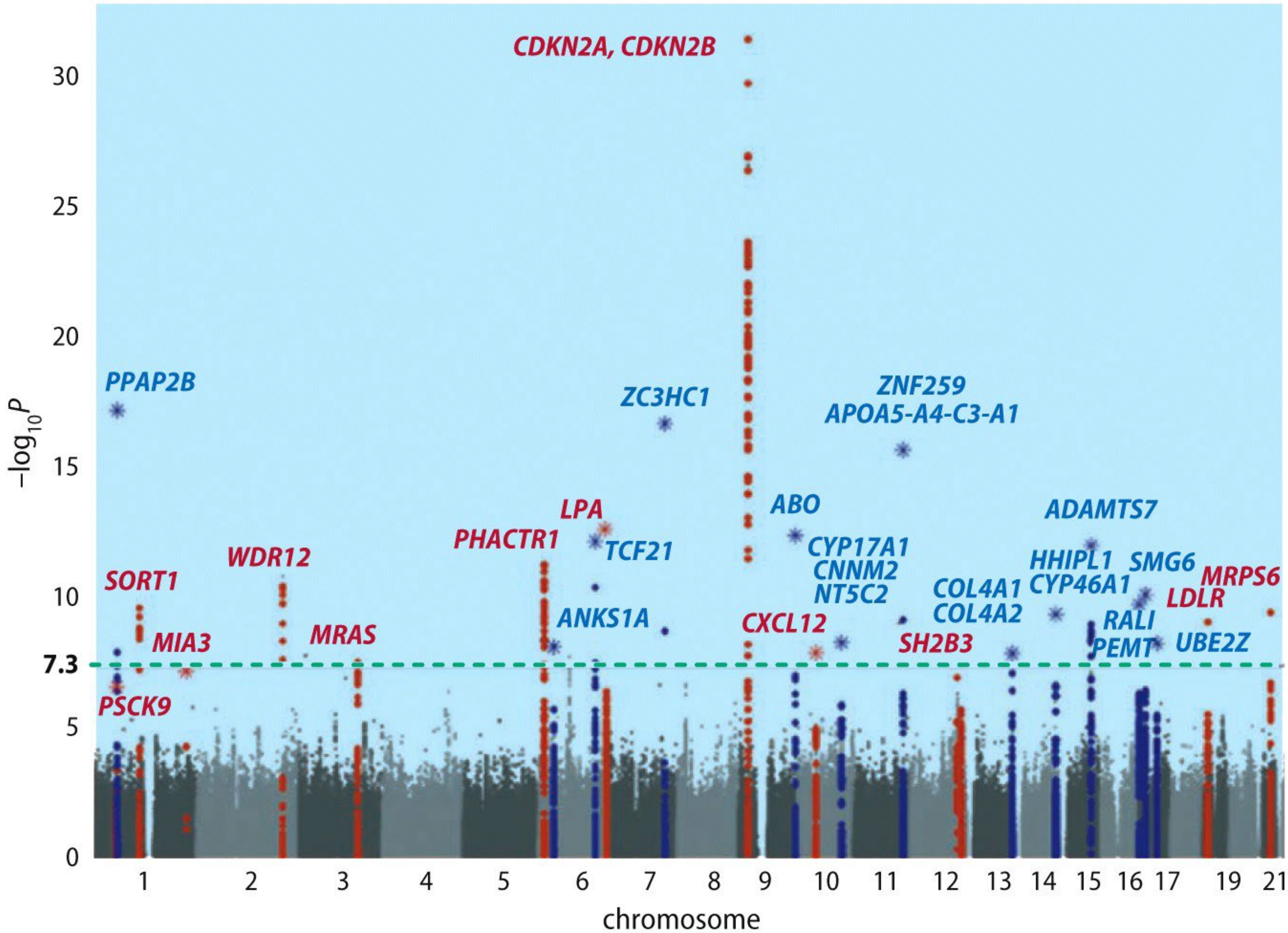
  (Inflated heritability)

# Manhatten plot

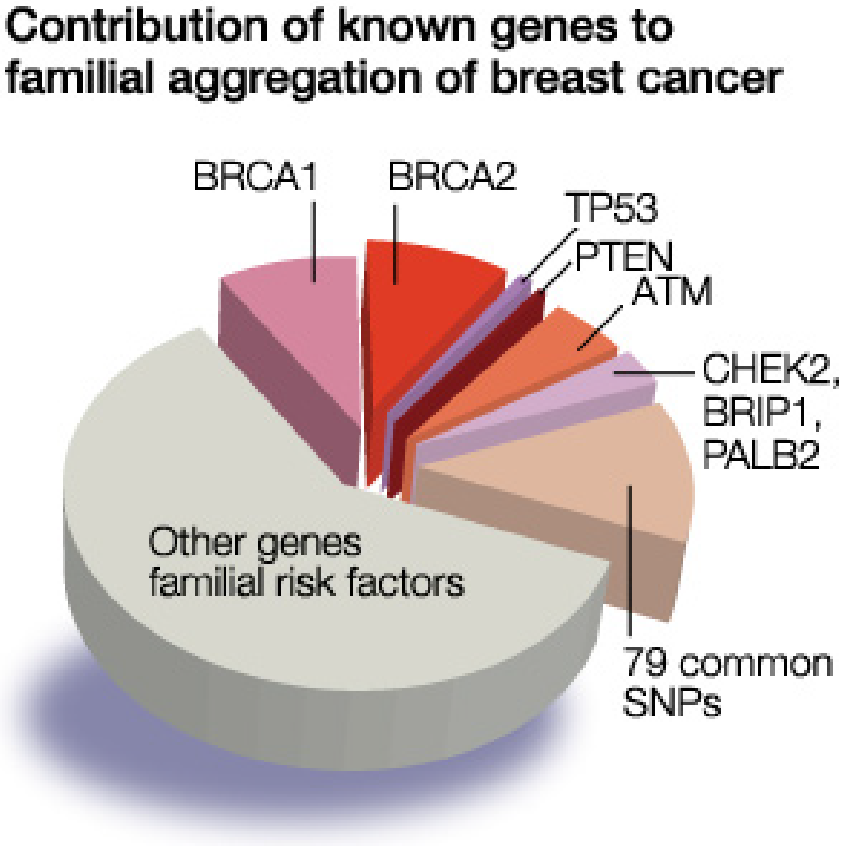
# Manhatten plot coronary artery disease

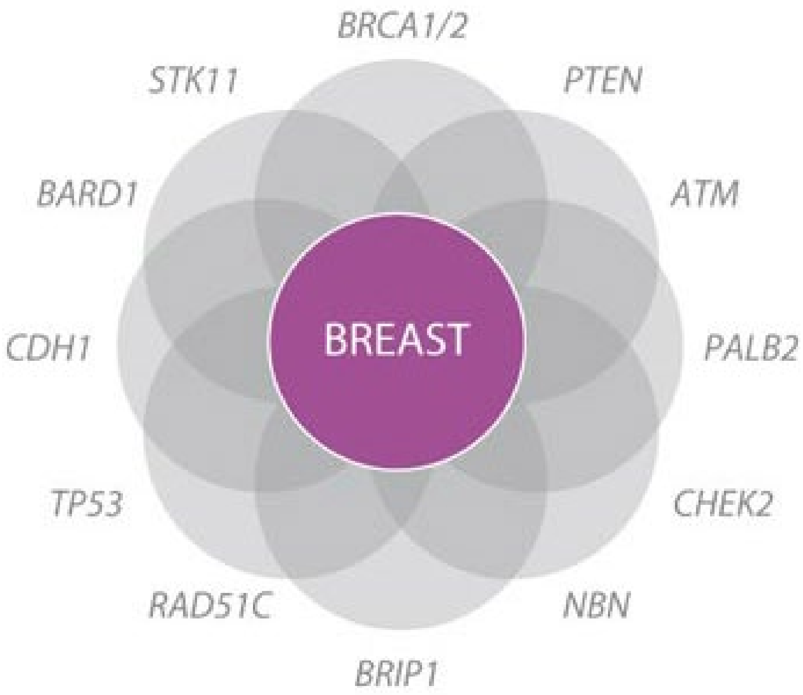


# Manhatten plot coronary artery disease

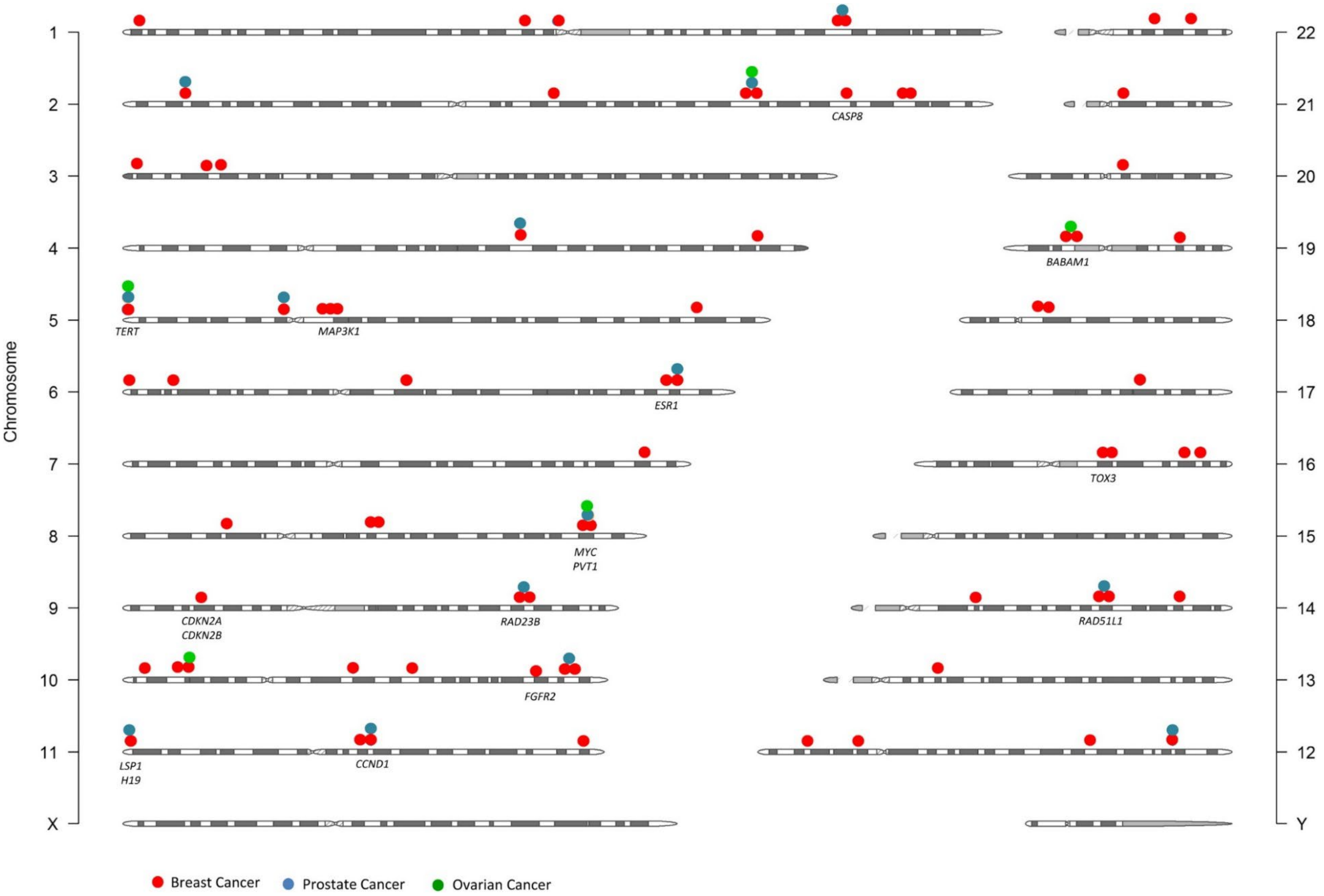


# Breast cancer susceptibility

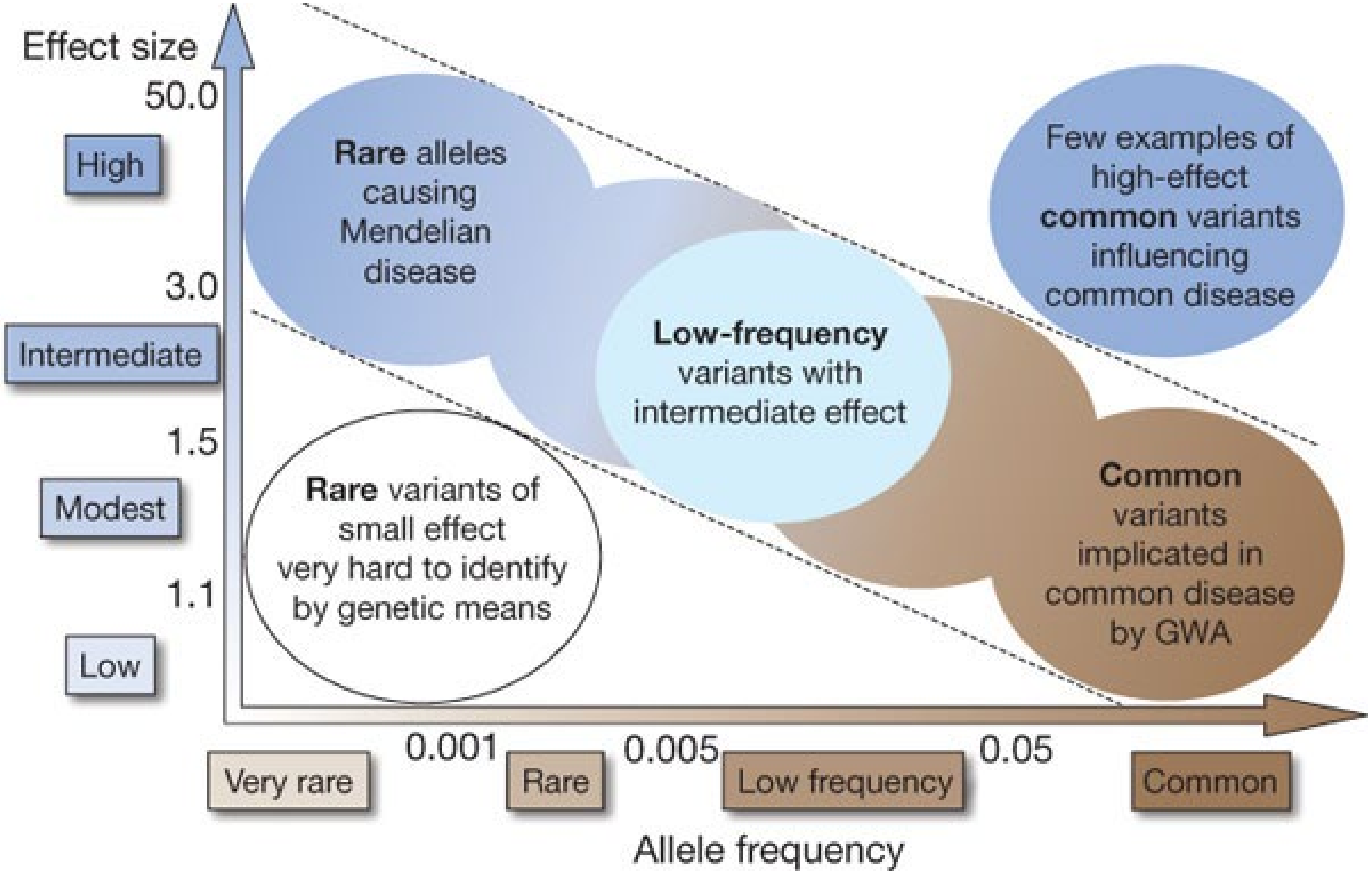




# Common breast cancer susceptibility loci



*The American Journal of Pathology* 2013 183, 1038-1051 odds ratio


Effect size
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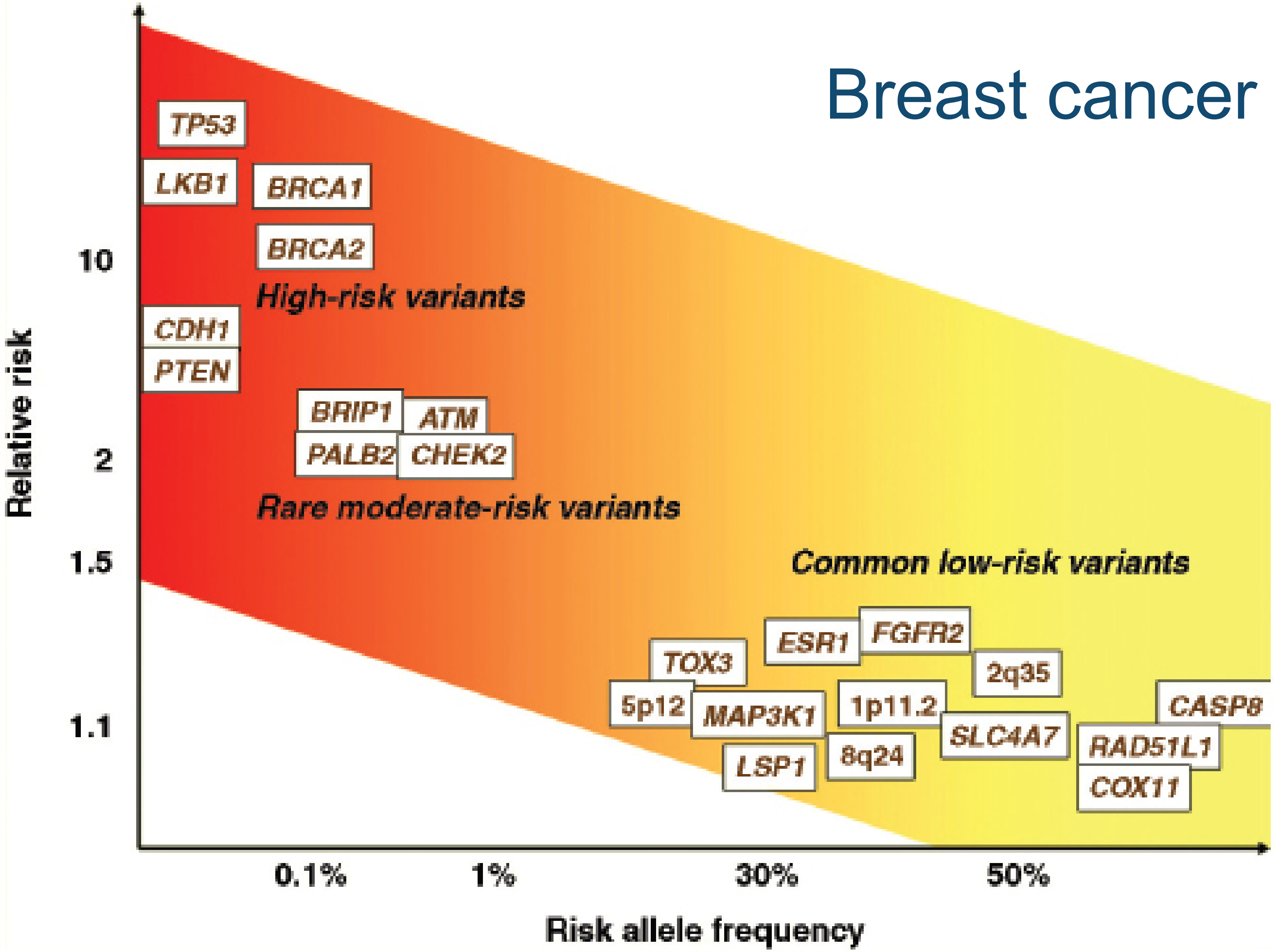
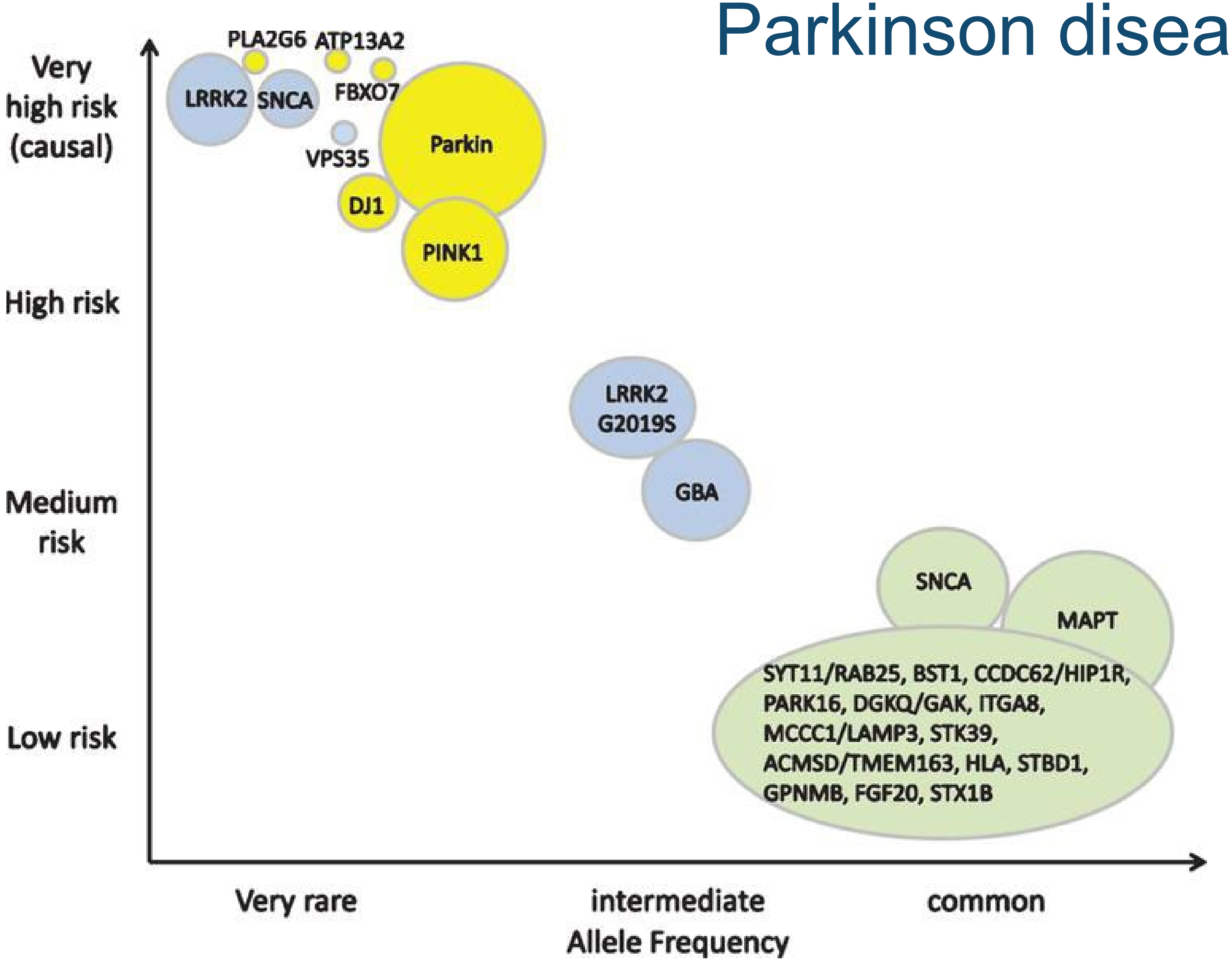
Rare alleles causing Mendelian disease
Low-frequency variants with intermediate effect
Few examples of high-effect common variants influencing common disease
Rare variants of small effect very hard to identify by genetic means
Common variants implicated in common disease by GWA
0.001
0.005
0.05
Very rare
Rare
Low frequency
Common
Allele frequency

Manolio *et al.* *Nature* **461**, 747-753 (2009)

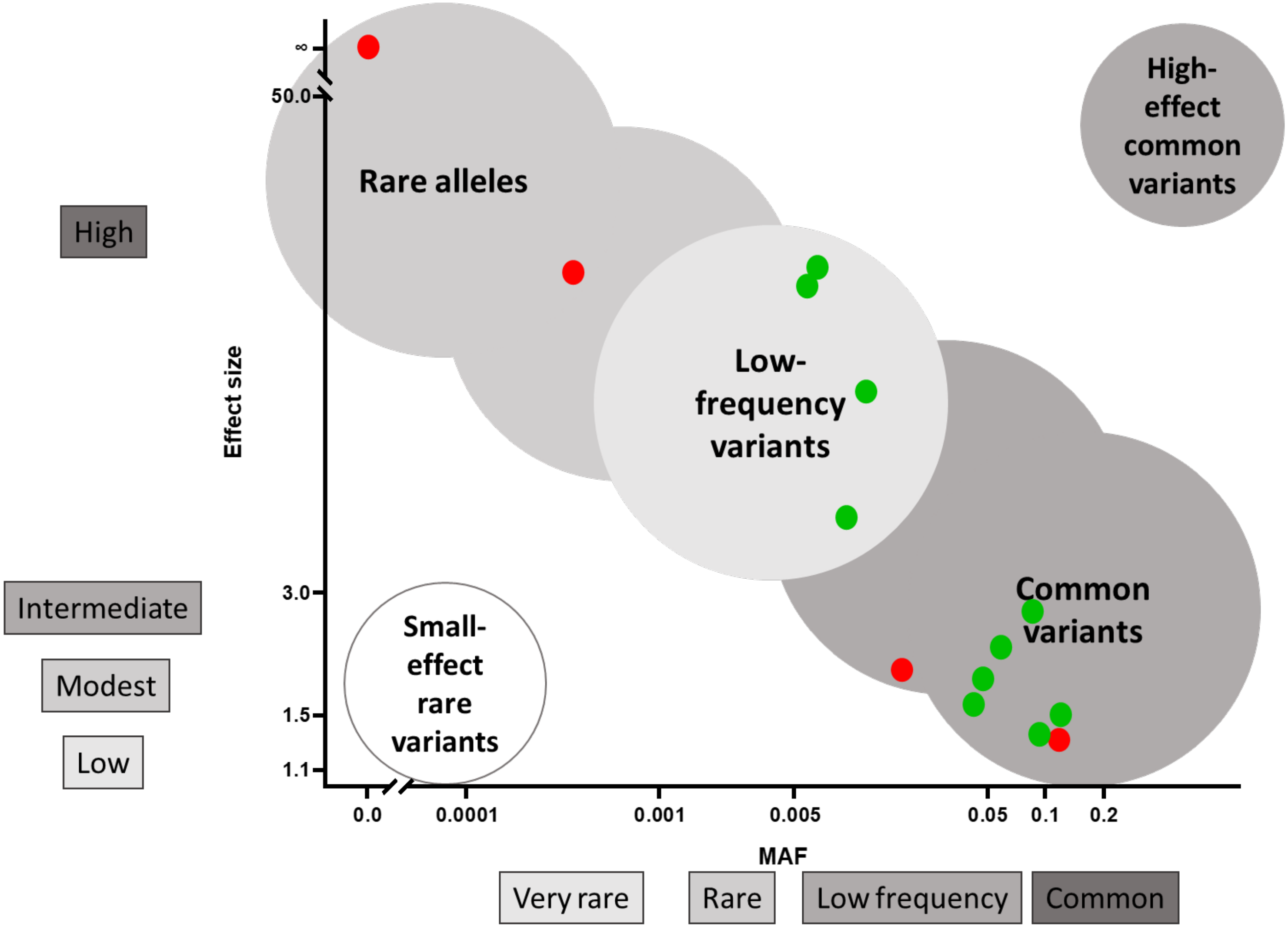
# Breast cancer



# Parkinson disease

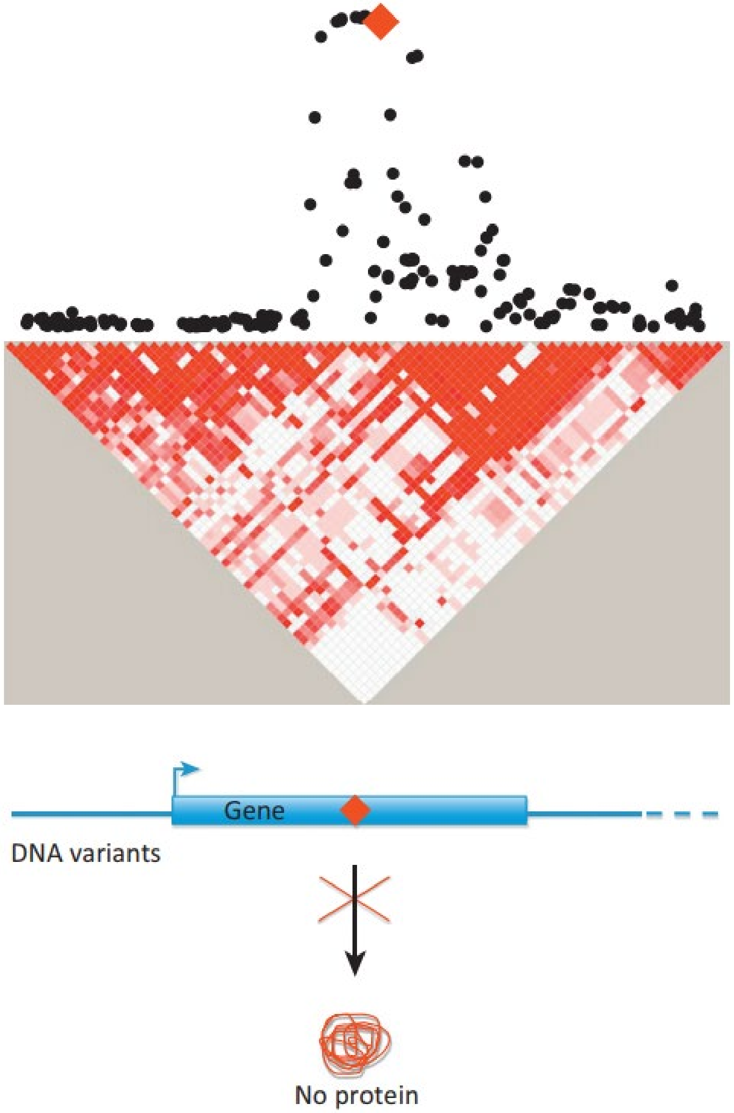


# Otosclerosis: association for ACAN gene variants



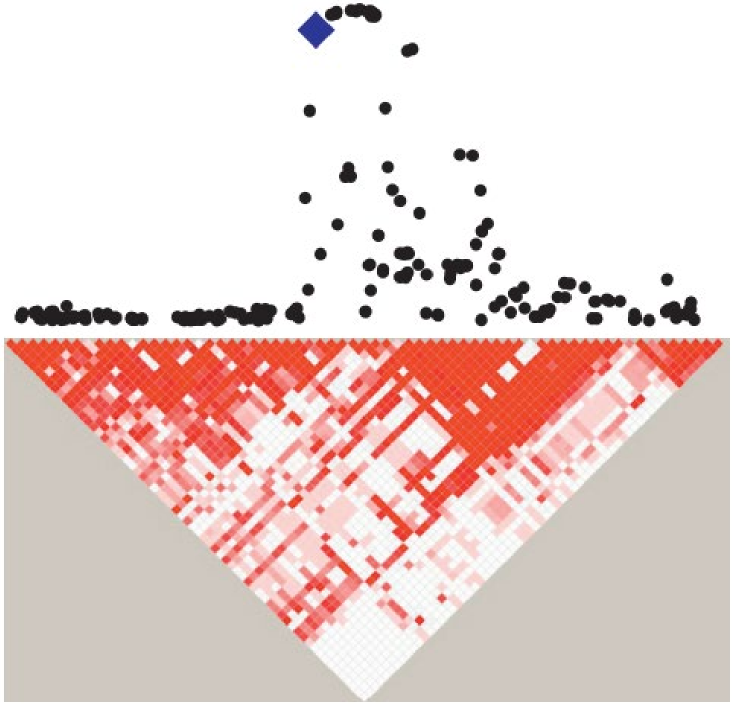
# How do genetic variants exert an effect?

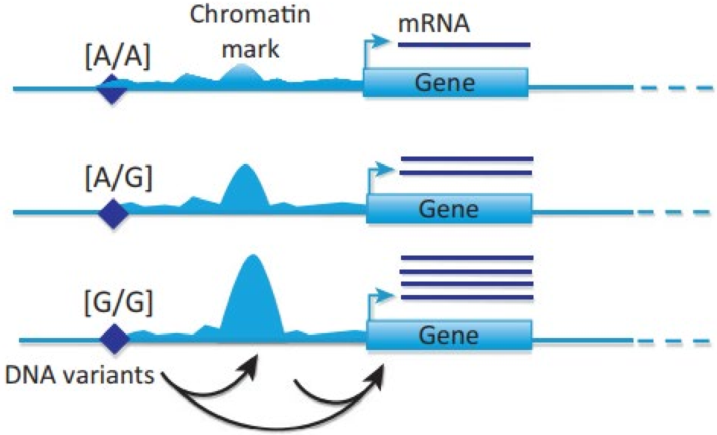
- Effect on the protein
- But … many associations are found outside coding regions



# How do genetic variants exert an effect?

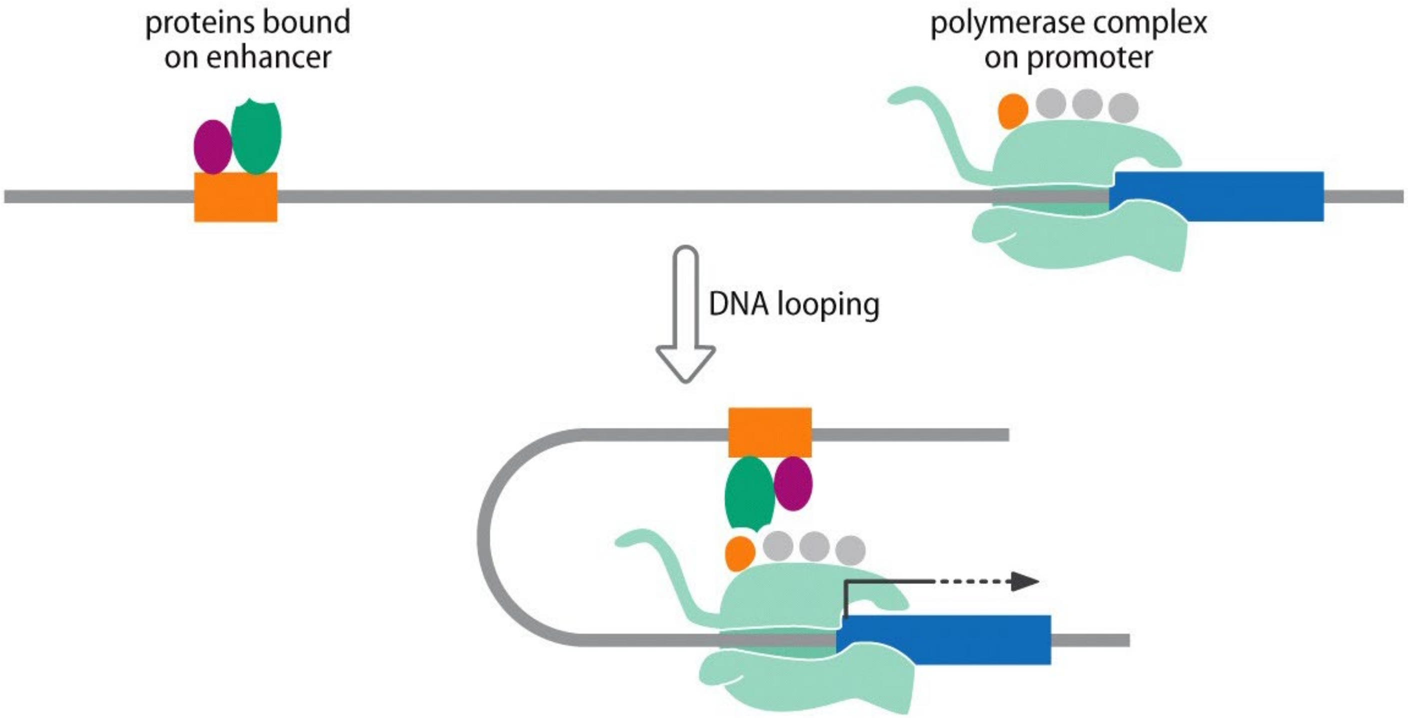
- Gene regulation
- TAD domain interactions



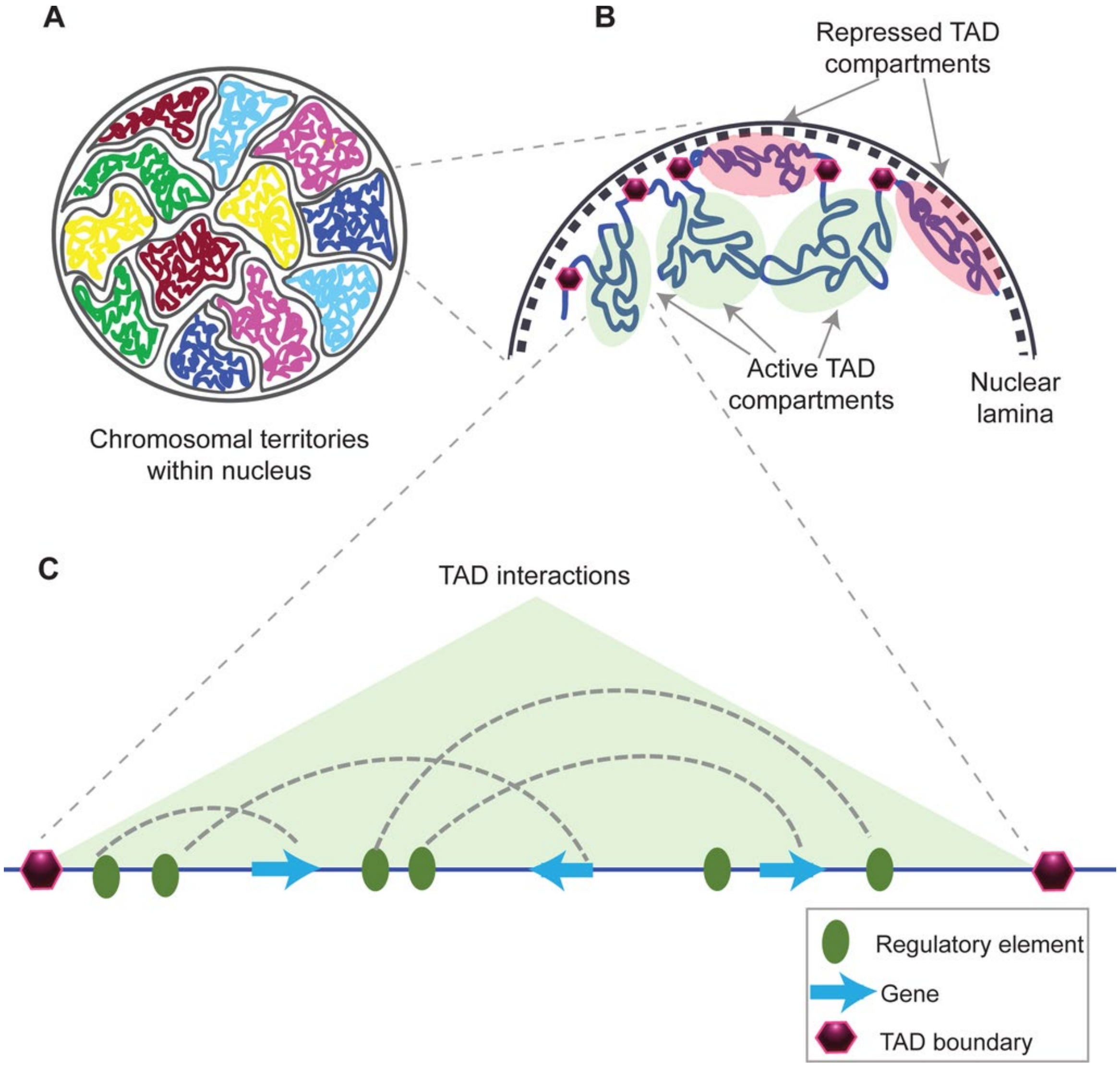


# Gene regulation

- Transcription factors (trans acting factors)
- TF bind DNA sequences (cis acting factors) (enhancers, silencers, promoters, …)

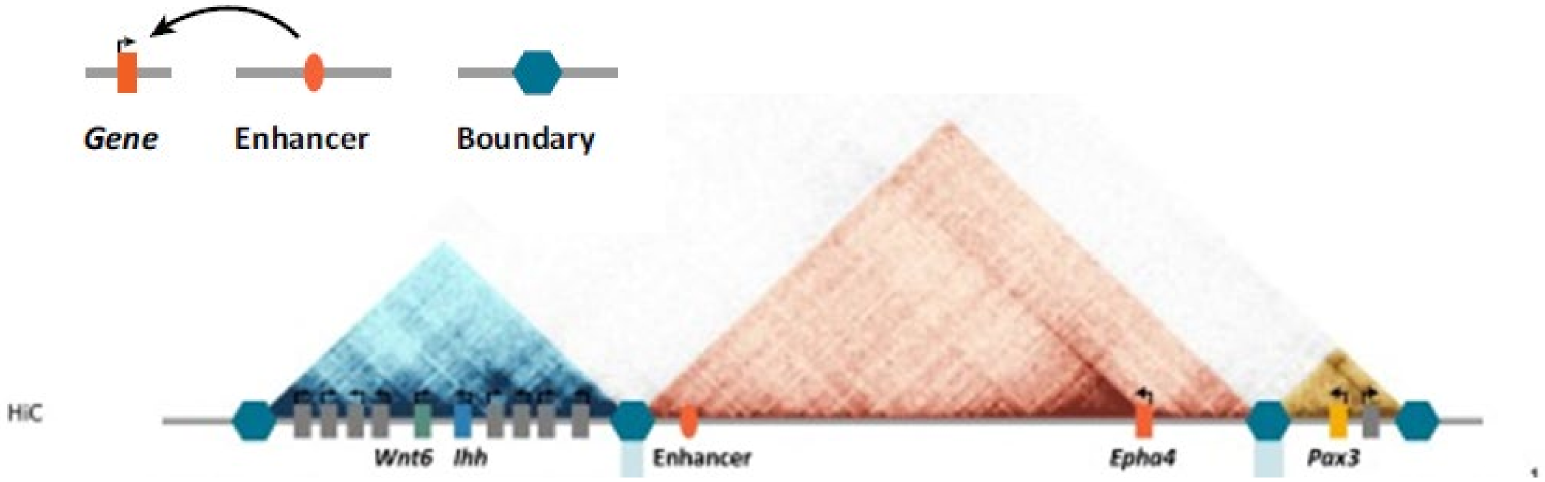


# Topologically associating domains (TADs)



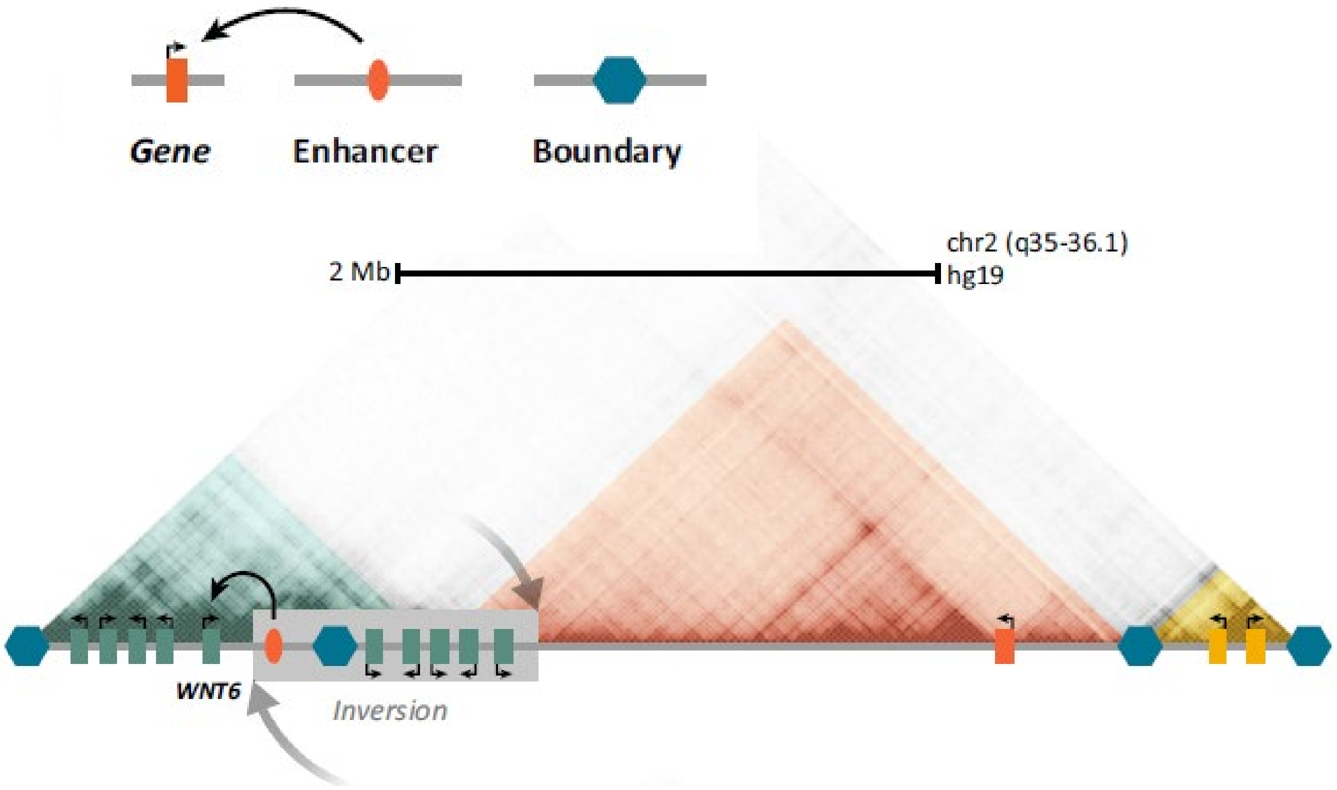
# Topologically associating domains (TADs)

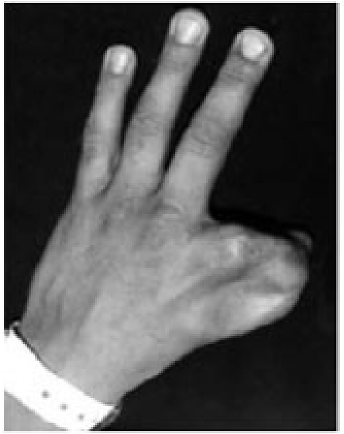
- Genomic region that limits promotor enhancer interactions
- Delimited by boundaries
- Evolutionary conserved



# Topologically associating domains (TADs)

Wrong expression of WNT6 by mislocalisation of enhancers of a neighbouring gene leads to syndactyly

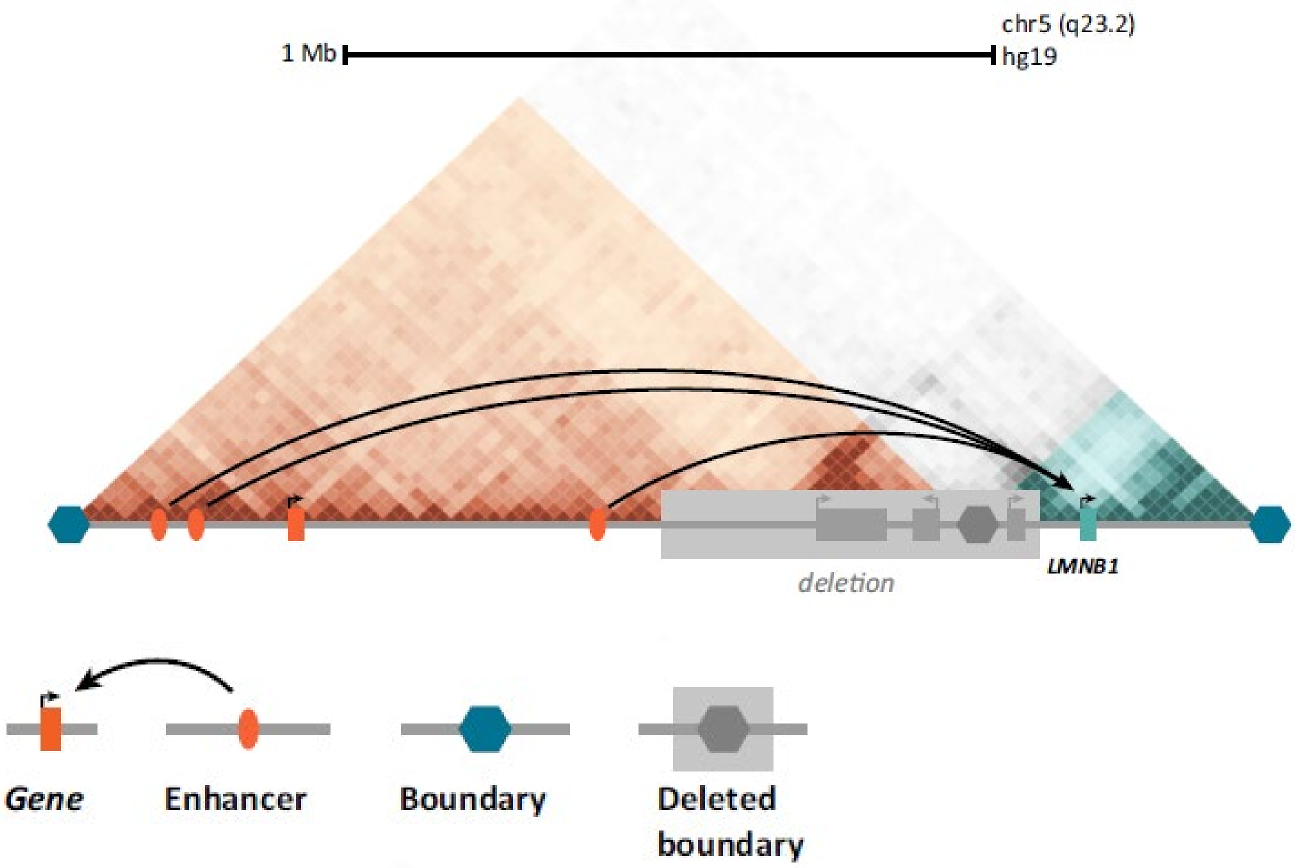


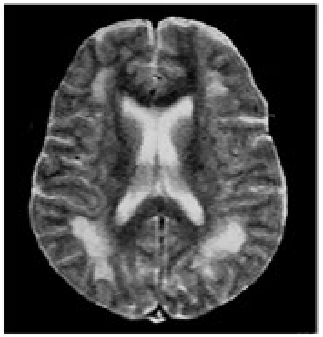


F-syndrome

# Topologically associating domains (TADs)

Overexpression of LMNB1 because of a deletion of a boundary leads to ADLD




Adult-onset demyelinating leukodystrophy