Statistical analysis of genome-wide association (GWAS) data

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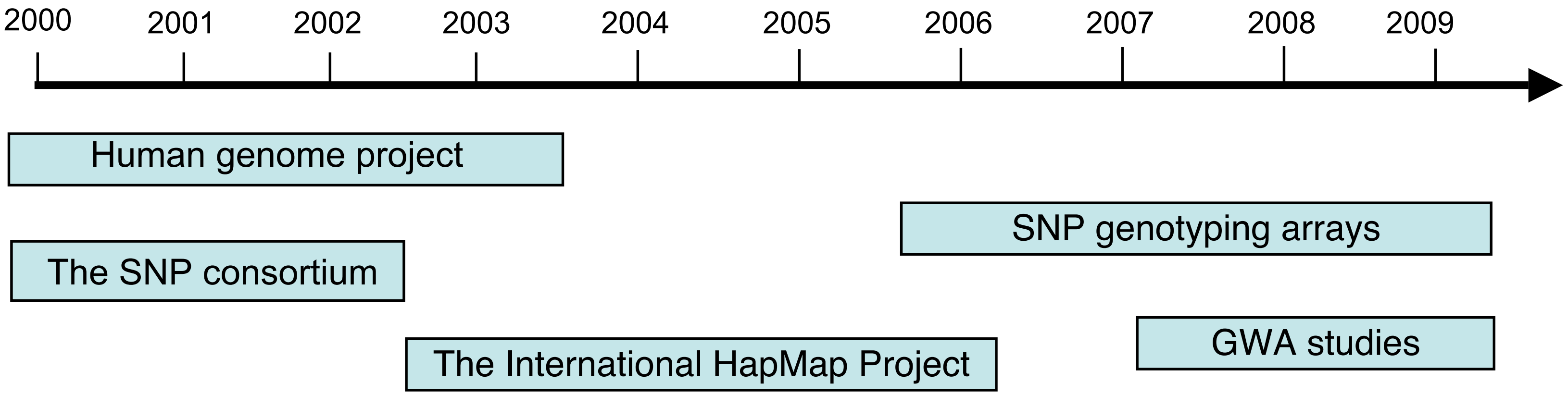
Outline

- Introduction
- Confounding variables and linkage disequilibrium
- Statistical methods to test for association in case-control GWA studies
  - Allele counting chi-square test
  - Logistic regression
- Multiple testing and power
- Example: GWAS for multiple sclerosis (MS)
  - Data cleaning / quality control
  - Results

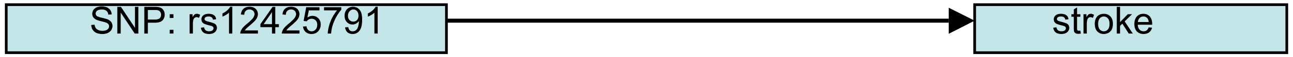
GWA studies have been very successful since 2007

- Prior to the advent of GWA studies, there was very little success in identifying genetic risk factors for complex multifactorial diseases
- GWA studies have identified over 200 separate associations with various complex diseases in the past two years
- “Human Genetic Variation” hailed as “Breakthrough of the Year” by Science magazine in 2007



This talk: case-control GWA studies

- Obtain DNA from people with disease of interest (cases) and unaffected controls
- Run each DNA sample on a SNP chip to measure genotypes at 300,000-1,000,000 SNPs in cases and controls
- Identify SNPs where one allele is significantly more common in cases than controls
  - The SNP is *associated* with disease



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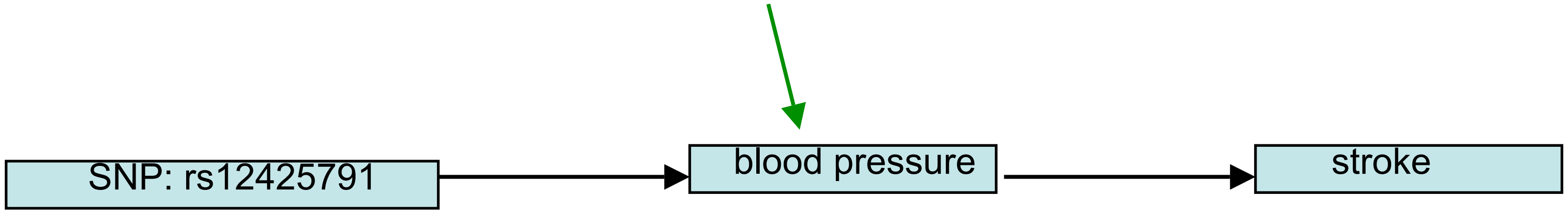
  - The SNP is *associated* with disease
- Alternative strategy (Peter Visscher’s talk):
  test for association between SNPs and a quantitative trait that underlies the disease (*endophenotype*)



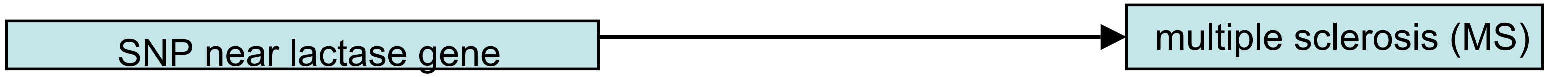
SNP: rs12425791

blood pressure

stroke

Association does not imply causation

- Suppose that genotypes at a particular SNP are significantly associated with disease
- This may be because the SNP is associated with some other factor (a *confounder*), which is associated with disease but is not in the same causal pathway



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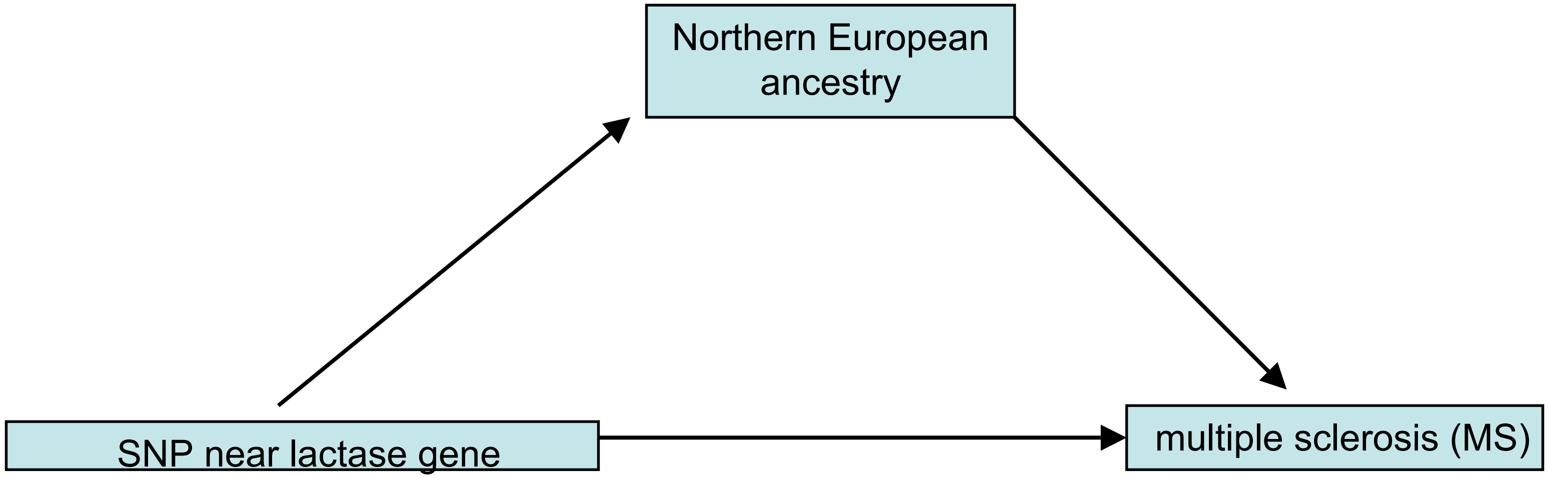
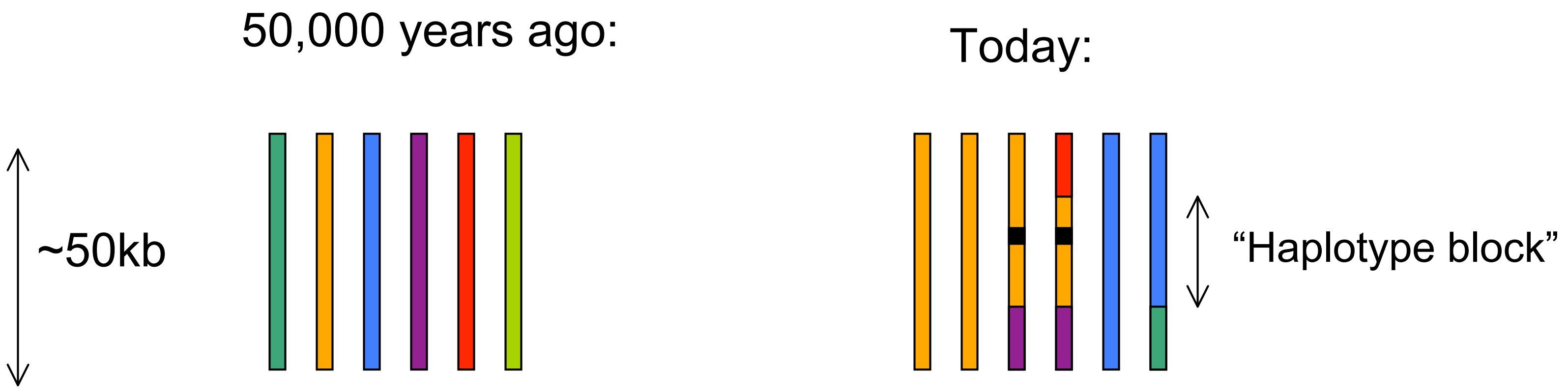


Association does not imply causation

- Suppose that genotypes at a particular SNP are significantly associated with disease
- This may be because the SNP is associated with some other factor (a *confounder*), which is associated with disease but is not in the same causal pathway
- Possible confounders of genetic associations:
  - Ethnic ancestry
  - Genotyping batch, genotyping centre
  - DNA quality
- Environmental exposures in the same causal pathway
  - Nicotine receptors --> smoking --> lung cancer
    Hung et al, Nature 452: 633 (2008) + other articles in same issue
  - Alcohol dehydrogenase genes --> alcohol consumption --> throat cancer
    Hashibe et al, Nature Genetics 40: 707 (2008)

Helpful confounding: linkage disequilibrium

Linkage disequilibrium (LD) is the non-independence of alleles at nearby markers in a population because of a lack of recombinations between the markers



50,000 years ago:

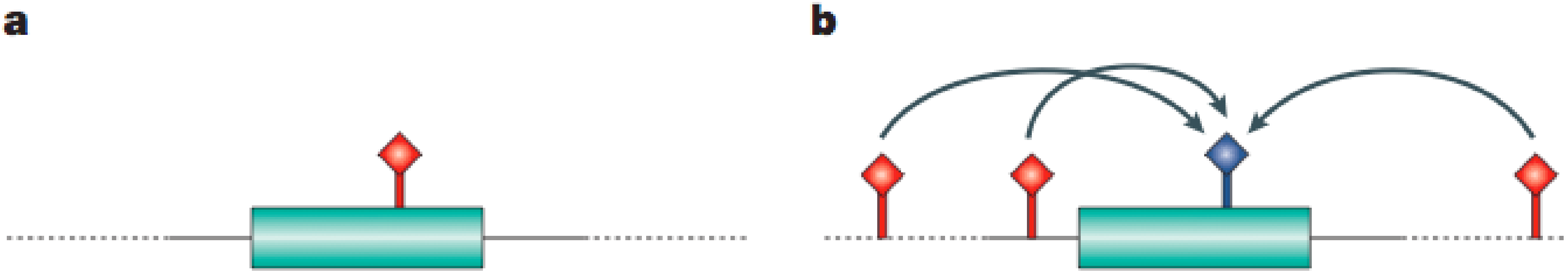
Today:

~50kb

“Haplotype block”

Direct and indirect association testing

Hirschhorn and Daly: Nature Reviews Genetics 6: 95 (2005)



Direct association

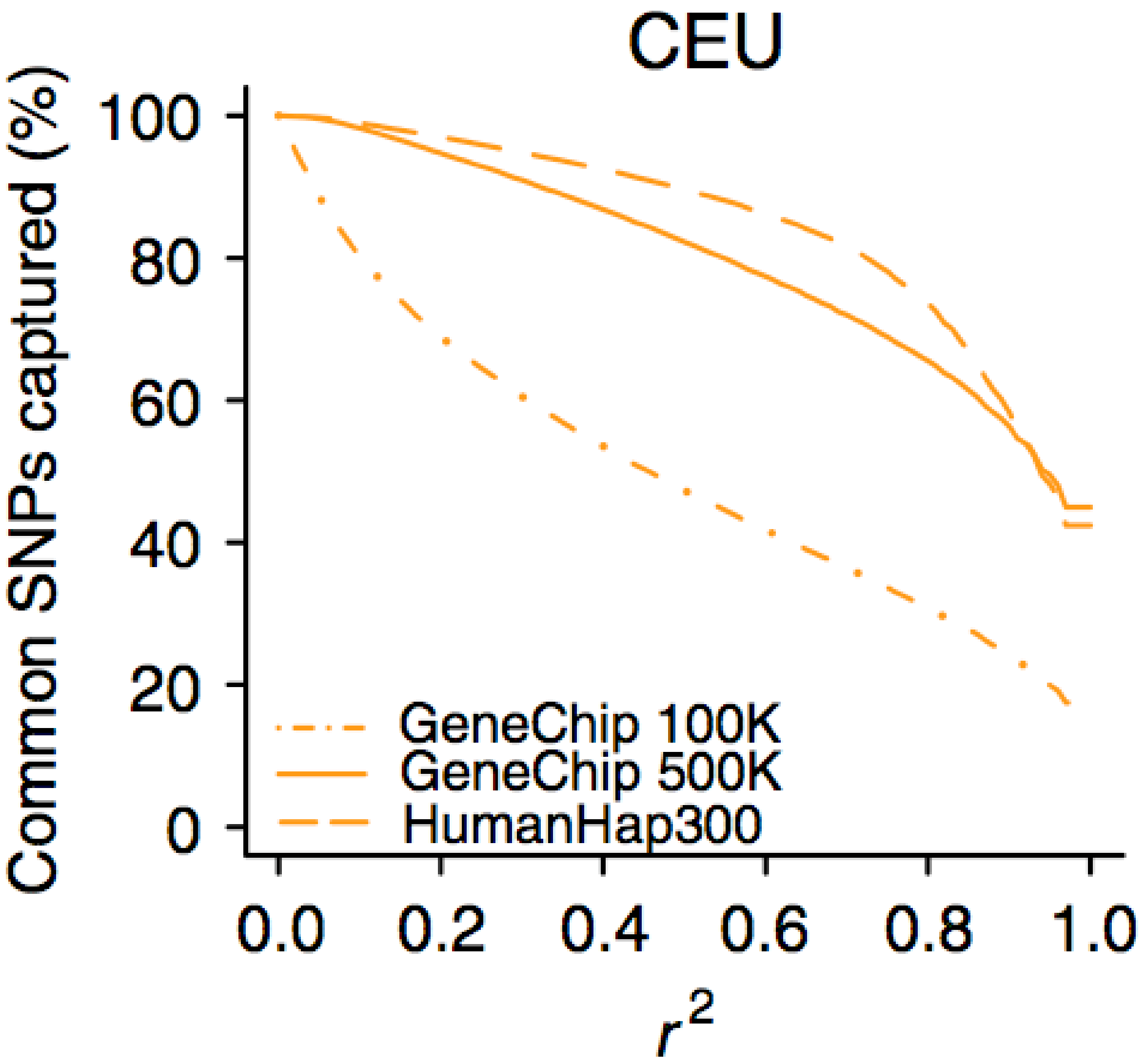
Indirect association

Functional SNP is genotyped and an association is found

Functional SNP (blue) is not genotyped, but a number of other SNPs (red), in LD with the functional SNP, are genotyped, and an association is found for these SNPs

LD is helpful, because not all SNPs have to be genotyped

Pe’er et al: Nature Genetics 38: 663 (2006)



Allele counting to test for association between SNP genotype and case / control status

	GG	**GT**	**TT**	**Total**
Cases	r_0	r_1	r_2	R
Controls	s_0	s_1	s_2	S
Total	n_0	n_1	n_2	N

Observed allele counts

	G	**T**	**Total**
Cases	$2r_0+r_1$	r_1+2r_2	$2R$
Controls	$2s_0+s_1$	s_1+2s_2	$2S$
Total	$2n_0+n_1$	n_1+2n_2	$2N$

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Expected allele counts

G	T
$2R(2n_0+n_1)/(2\mathrm{N})$	$2R(n_1+2n_2)/(2\mathrm{N})$
$2S(2\mathrm{n}_0+\mathrm{n}_1)/(2\mathrm{N})$	$2S(n_1+2n_2)/(2\mathrm{N})$

Allele counting to test for association between SNP genotype and case / control status

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$2S(2\mathrm{n}_0+\mathrm{n}_1)/(2\mathrm{N})$	$2S(n_1+2n_2)/(2\mathrm{N})$

Chi-square test for independence of rows and columns (null hypothesis):

$$\sum \frac{(\mathrm{Obs} - \mathrm{Exp})^2}{\mathrm{Exp}} \sim \chi^2 \text{ with 1 df}$$

PLINK `--assoc` option

Other options (e.g. dominant/recessive models)
`--model`

The odds ratio: a measure of effect size

Odds of an event occurring = Pr(event occurs) / Pr(event doesn’t occur)
= Pr(event occurs) / [1 - Pr(event occurs)]

Allele counts

	G	**T**
Cases	*a*	*b*
Controls	*c*	*d*

Consider all the G alleles in the sample, and pick one at random.
The odds that the G allele occurs in a case: a/c

Consider all the T alleles in the sample, and pick one at random.
The odds that a T allele occurs in a case: b/d

$$\textit{odds ratio} = \frac{\text{odds that G allele occurs in a case}}{\text{odds that T allele occurs in a case}} = \frac{a/c}{b/d} = \frac{a\,d}{b\,c}$$

Interpretation of the odds ratio

	G	**T**
Cases	*a*	*b*
Controls	*c*	*d*

odds ratio (OR) $= \dfrac{\text{odds that G allele occurs in a case}}{\text{odds that T allele occurs in a case}} = \dfrac{a\,d}{b\,c}$

OR = increase in odds of being a case for each additional G allele

OR = 1: no association between genotype and disease
OR > 1: G allele increases risk of disease
OR < 1: T allele increases risk of disease

If the disease is rare (e.g. ~0.1% for MS), the odds ratio is roughly equal to the *genotype relative risk (GRR)*:
the increase in risk of disease conferred by each additional G allele

e.g. if OR = 1.2,
Pr(MS I TT) = 0.1%   Pr(MS I GT) = 0.12%   Pr(MS I GG) = 0.144%

Logistic regression: more flexible analysis for GWA studies

- Similar to linear regression, used for binary outcomes instead of continuous outcomes
- Let Y_i be the phenotype for individual i

  $Y_i = 0$ for controls

  $Y_i = 1$ for cases
- Let X_i be the genotype of individual i at a particular SNP

TT	$X_i = 0$
GT	$X_i = 1$
GG	$X_i = 2$

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- Basic logistic regression model

  Let $p_i = \mathrm{E}(Y_i \mid X_i)$, expected value of pheno given geno

  Define logit(p_i) = $\log_e[p_i / (1 - p_i)]$

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Test whether β_1 differs significantly from zero:
roughly equivalent to allele counting chi-square test

Estimate of odds ratio: $\exp(\beta_1)$

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- Add extra terms to adjust for potential confounders: e.g. ethnicity, genotyping batch, genotypes at other SNPs

  Let $p_i = \mathrm{E}(Y_i \mid X_i, C_i, D_i, \ldots)$

$$\mathrm{logit}(p_i) \sim \beta_0 + \beta_1 X_i + \beta_2 C_i + \beta_3 D_i + \ldots$$

PLINK `--logistic`

Multiple testing

- Suppose you test 500,000 SNPs for association with disease
- Expect around 500,000 x 0.05 = 25,000 to have p-value less than 0.05
- More appropriate significance threshold

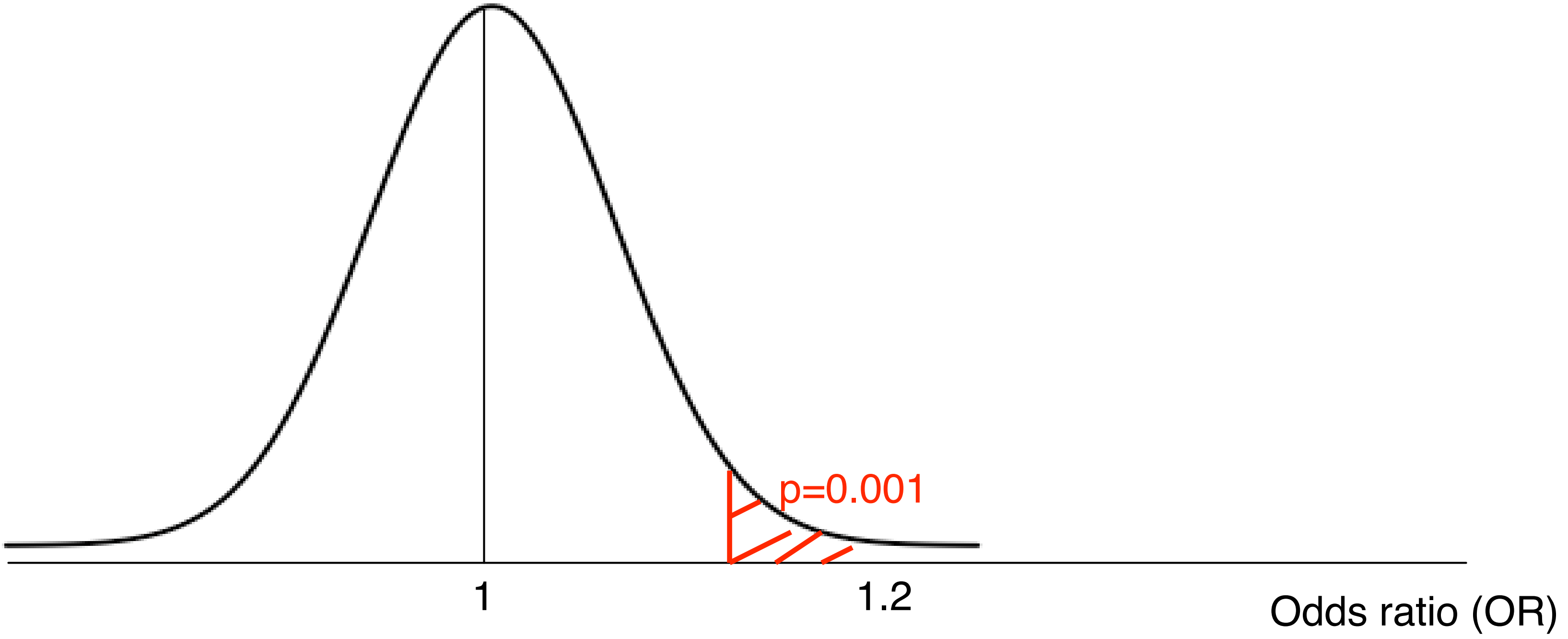
  $p = 0.05 / 500,000 = 10^{-7}$

  genome-wide significance
- In our MS GWAS we considered SNPs for follow-up if they had p-values less than 0.001
- To detect a smaller p-value need a larger study

The power to detect an association

- Suppose the G allele of a SNP has frequency 0.2. If each additional G allele increases odds of disease by 1.2, and 1618 cases and 3413 controls are genotyped, what is the *power* (chance) of detecting an association with significance p<0.001?

Null hypothesis: true OR=1
Observed OR from this distribution



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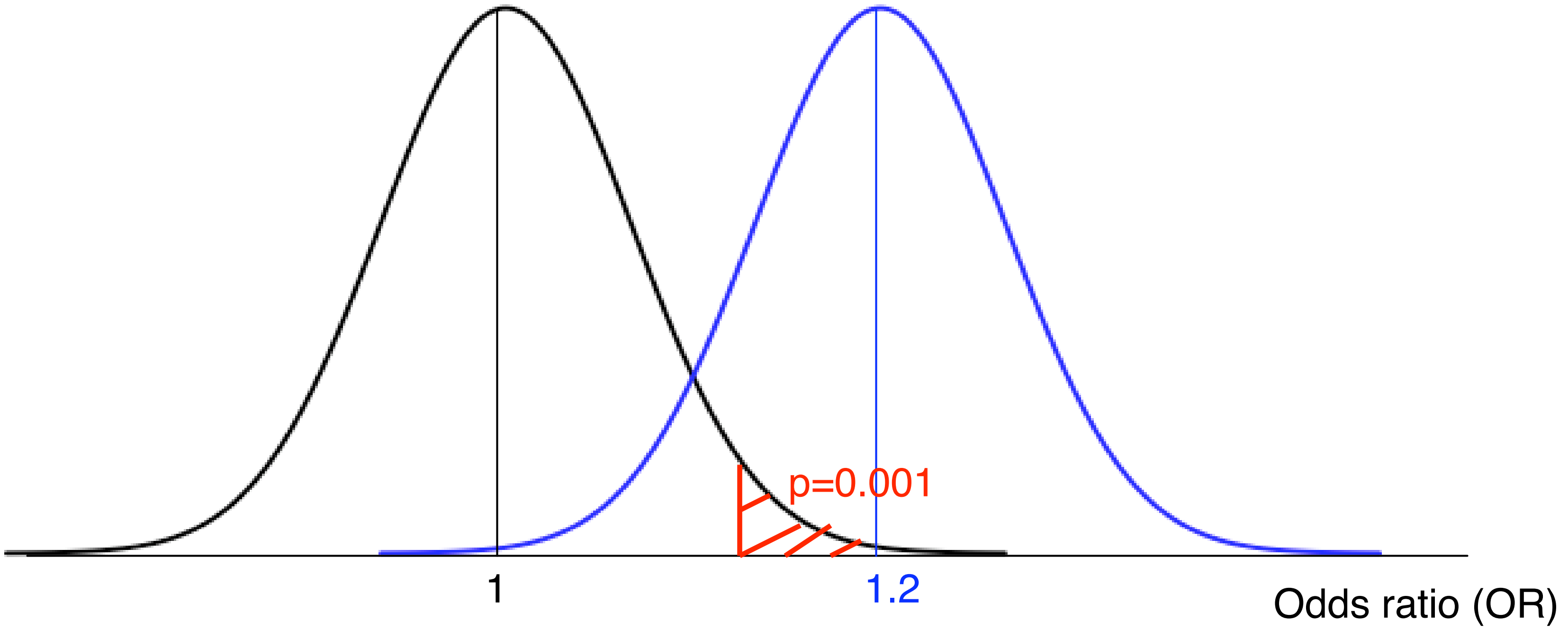

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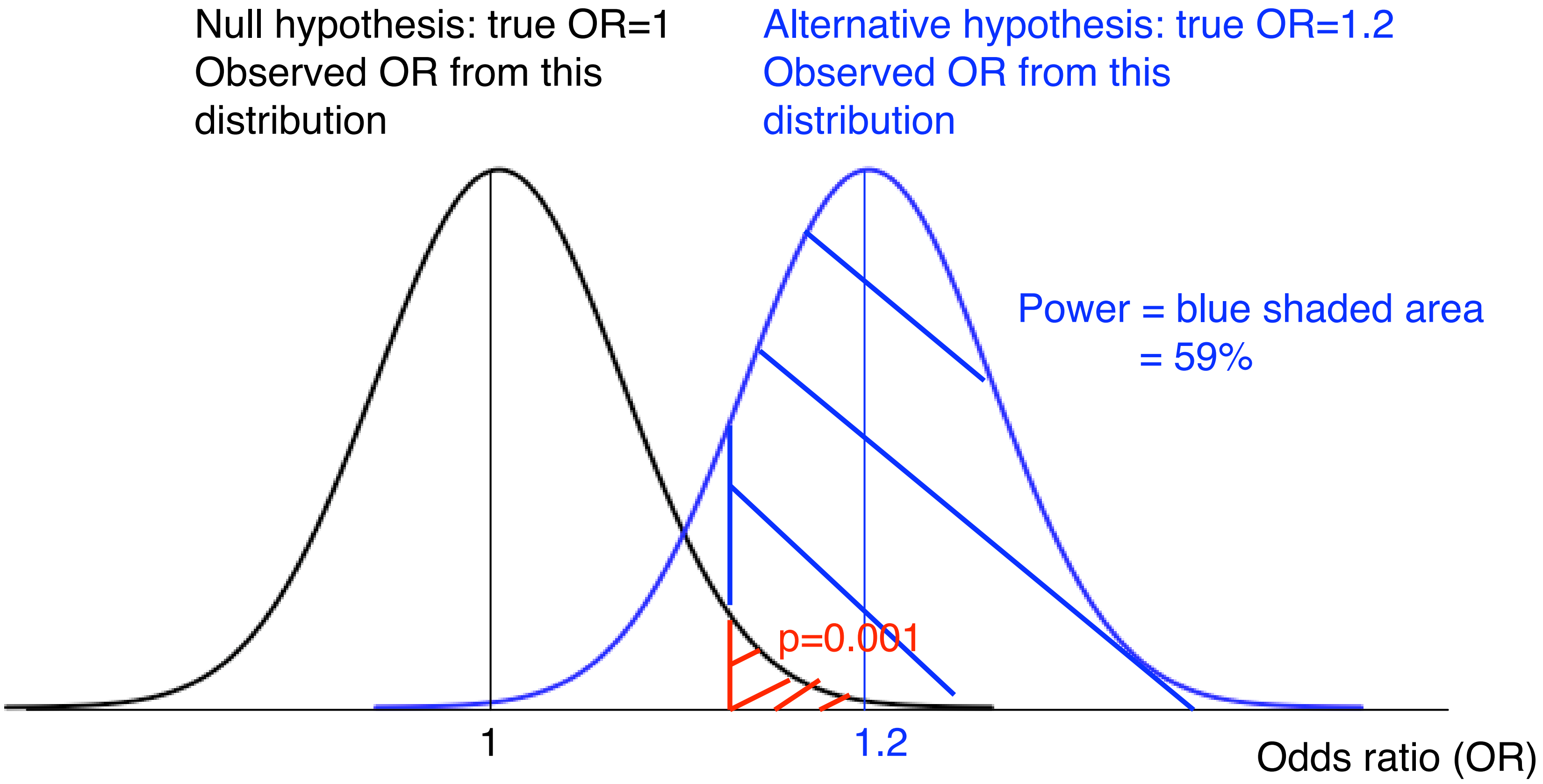


Effect of increasing sample size

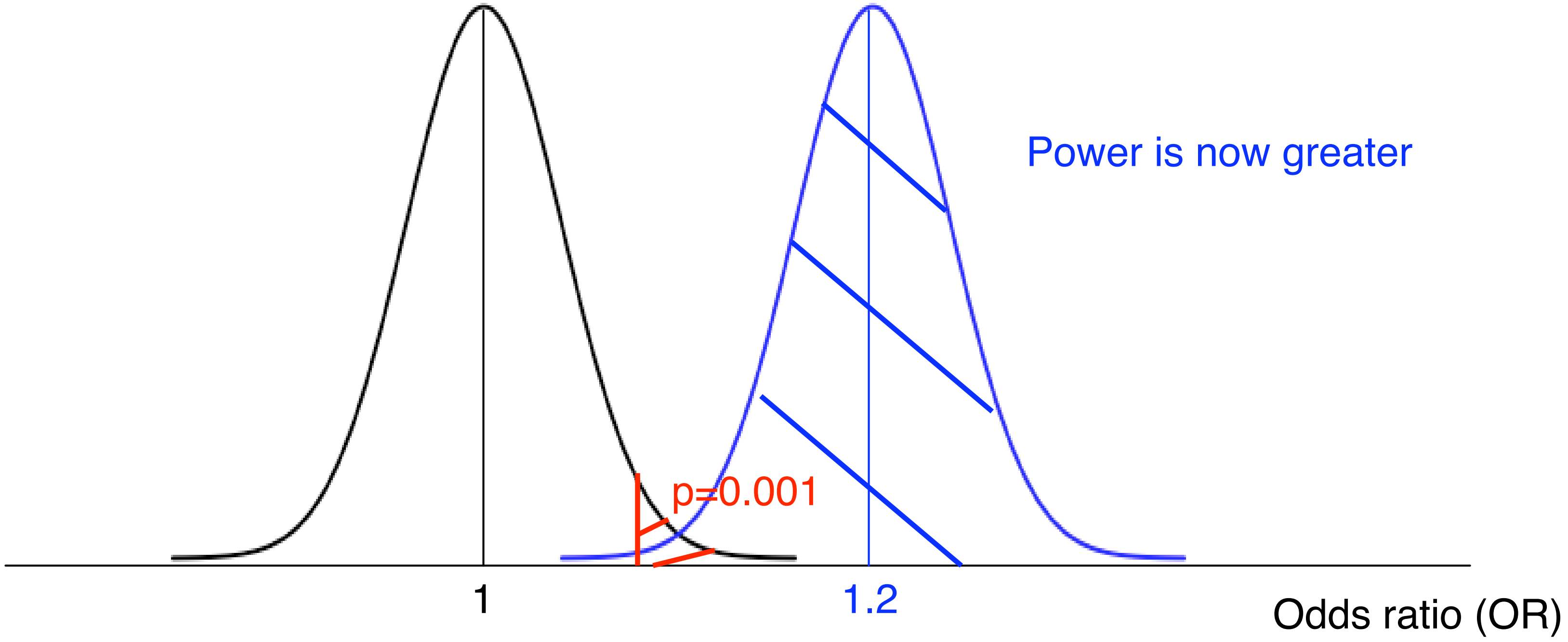
Observed OR tends to be closer to true OR (narrower distributions)

⇒ Null and alternative distributions become more separate

⇒ Power increases

Null hypothesis: true OR=1
Observed OR from this
distribution

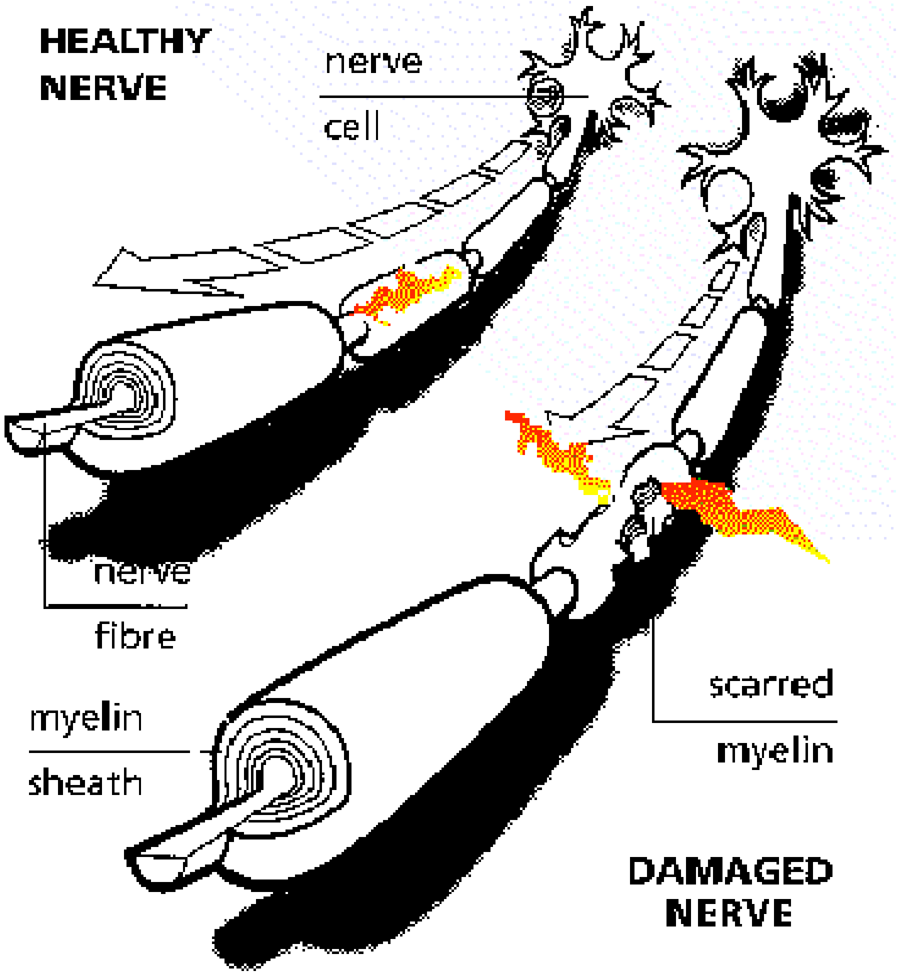
Alternative hypothesis: true OR=1.2
Observed OR from this
distribution



Power is now greater

p=0.001

Multiple sclerosis - degradation of myelin sheath around nerve fibres



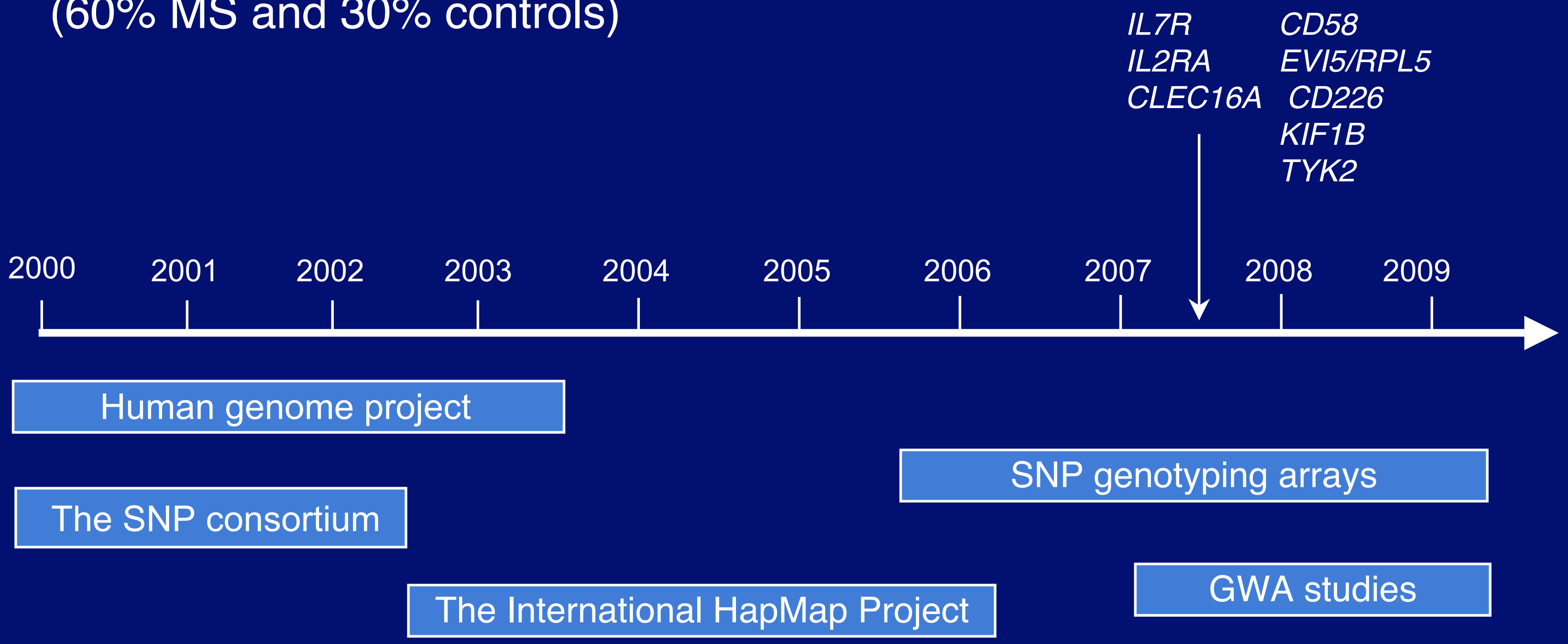
www.msif.org/en/about_ms/ demyelination.html

Multiple sclerosis

- neurodegenerative disease
- autoimmune attack on myelin sheaths around nerve cells
- more females affected than males (3:1)
- average age-at-onset ~30
- ~16,000 people with MS in Australia ($2 billion p.a.)
- no cure

Risk factors

- Epstein-Barr virus
- Exposure to infant siblings (Ponsonby et al, JAMA, 2005)
- Latitude gradient, childhood sun exposure (van der Mei et al, Lancet, 2003)
- Only genetic risk factor known before 2007 (first GWAS): HLA-DRB1*1501 discovered in 1972 (60% MS and 30% controls)



IL7R
IL2RA
CLEC16A

CD58
EVI5/RPL5
CD226
KIF1B
TYK2

2000 2001 2002 2003 2004 2005 2006 2007 2008 2009

Human genome project

The SNP consortium

The International HapMap Project

SNP genotyping arrays

GWA studies

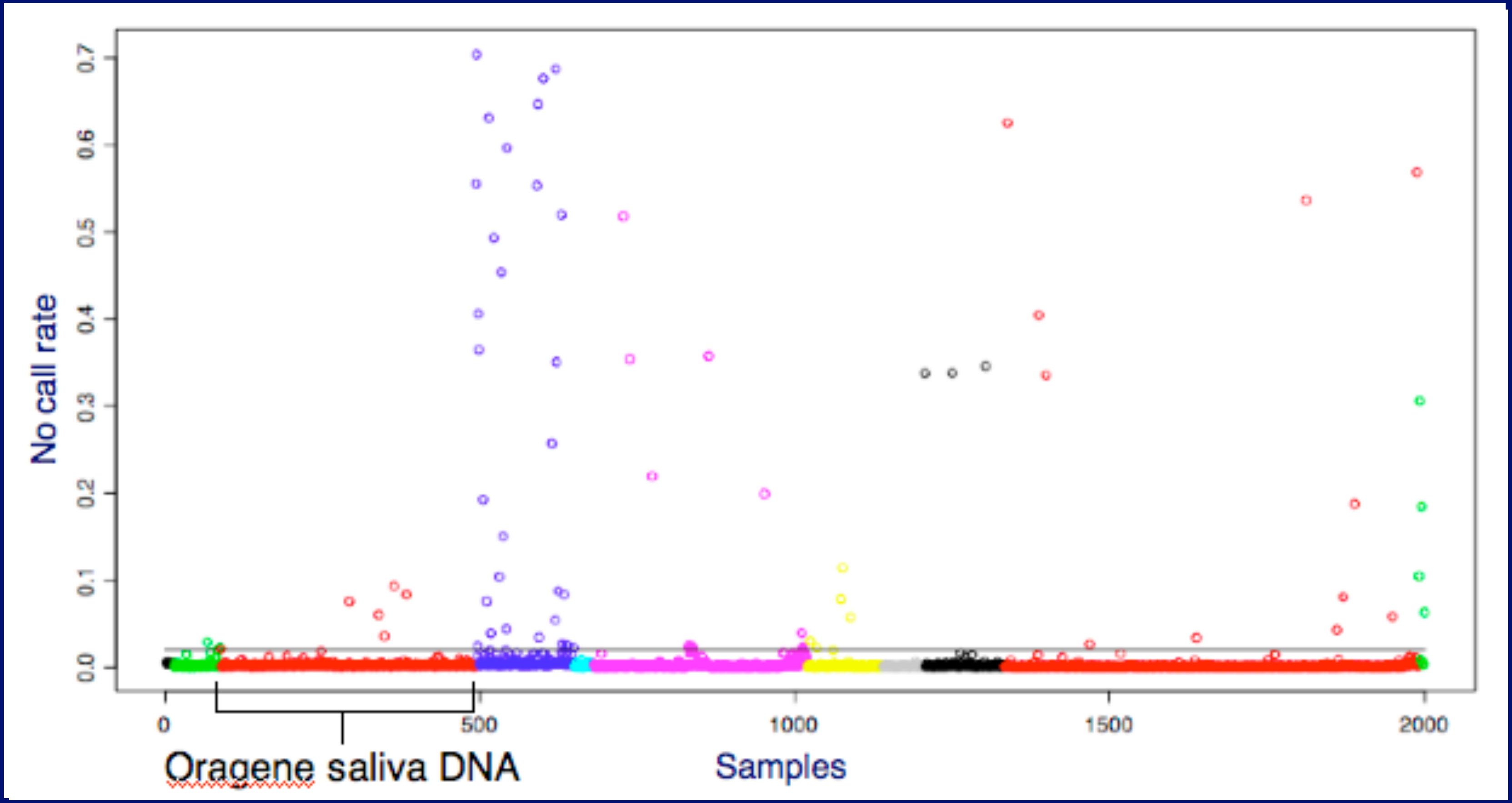
Australian and New Zealand MS GWAS

- Assemble collection of DNA samples (all states + NZ)
- Genotype 1952 MS cases from around Australia and New Zealand with Illumina 370CNV BeadChips
  (Patrick Danoy, Matt Brown, Diamantina Institute, UQ)
- Analyse GWAS data
  - Quality control (Devindri Perera, Menzies)
  - Impute genotypes at millions of other SNPs
    (Sharon Browning, Univ of Auckland)
  - Compare case genotypes with >3500 controls from the UK and US
    (publicly available data)
- Replication genotyping
  (Justin Rubio's lab, Howard Florey Institute, Univ of Melbourne)

Quality control - MS samples (PLINK)

- Start with 1952 samples
- Exclusions
  - Samples with >2% of SNPs not called 70 `--mind`

Genotype call rate

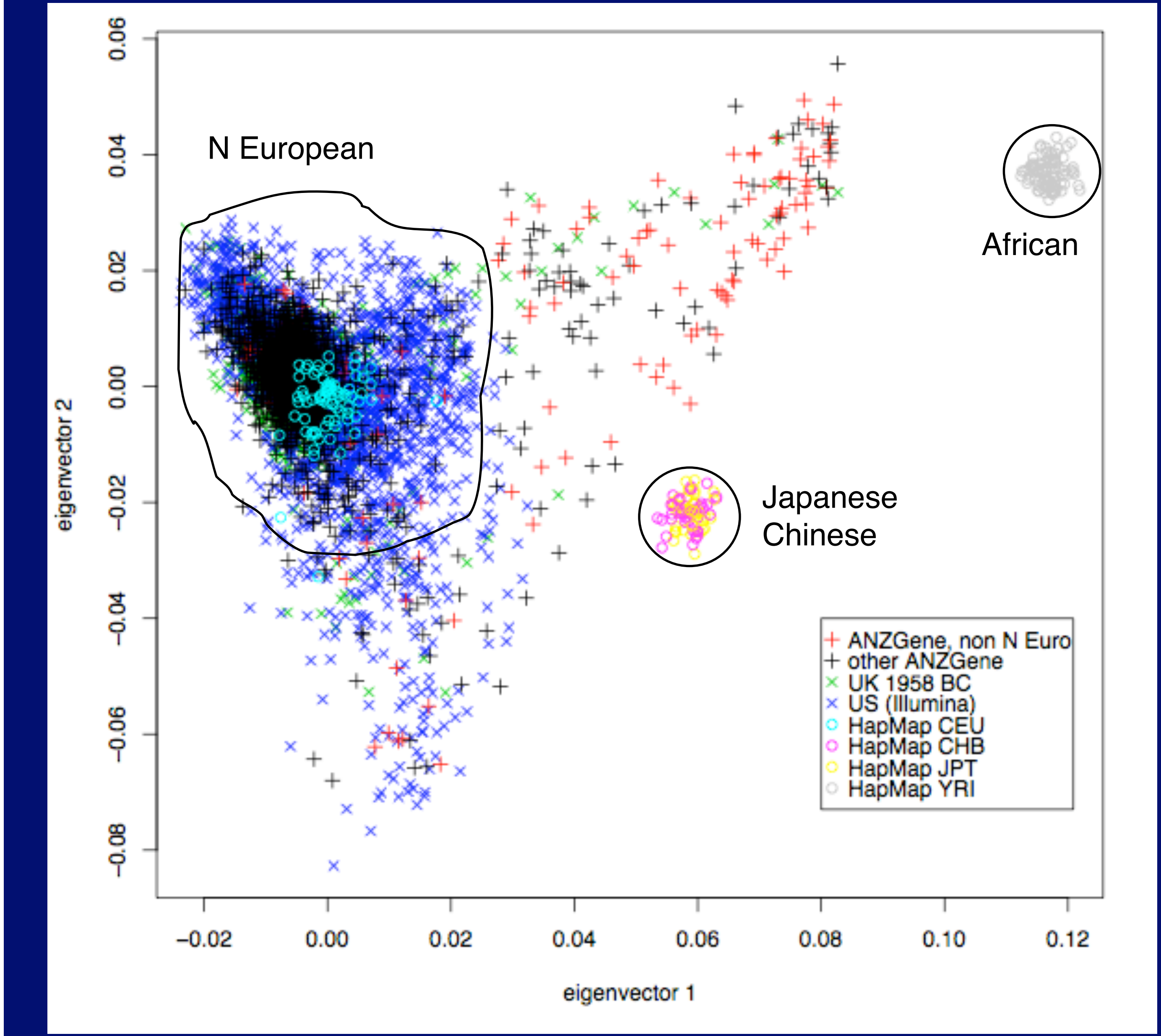


Quality control - MS samples (PLINK)

- Start with 1952 samples
- Exclusions

Samples with >2% of SNPs not called	70	`--mind`
Suspect batch of samples	128	
Uncertain phenotype	10	
Duplicates / relatives	88	`--genome`
Ancestry outliers	35	

Quality control - ethnicity



- Principal components analysis: EIGENSTRAT Price et al (2006). Nat Genet 38: 904

- Use an independent set of ~77,000 SNPs

  `--indep-pairwise`

- 178 outliers removed:
  - 35 MS
  - 143 controls

Quality control - MS samples

- Start with 1952 samples
- Exclusions

– Samples with >2% of SNPs not called	70	`--mind`
– Suspect batch of samples	128	
– Uncertain phenotype	10	
– Duplicates / relatives	88	`--genome`
– Ancestry outliers	35	
– Sex discrepancies	3	`--check-sex`

- Leaves 1618 samples

Quality control - SNPs

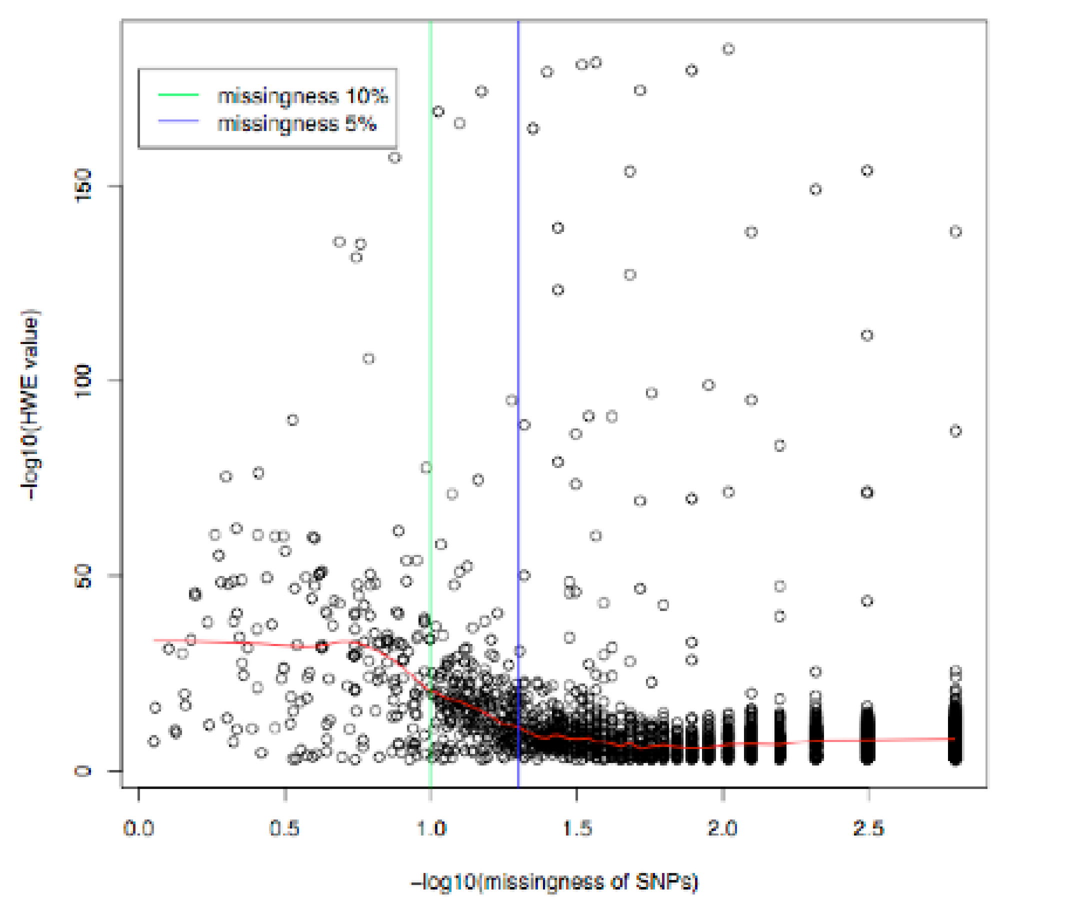
- Start with 310,504 SNPs in both case and control datasets
- Exclude SNPs

– Not called in >5% of samples	`--geno`
– In Hardy-Weinberg disequilibrium	`--hwe`
– Where one allele has frequency < 1%	`--maf`

- Leaves 302,098 SNPs

Choice of 5% no-call threshold

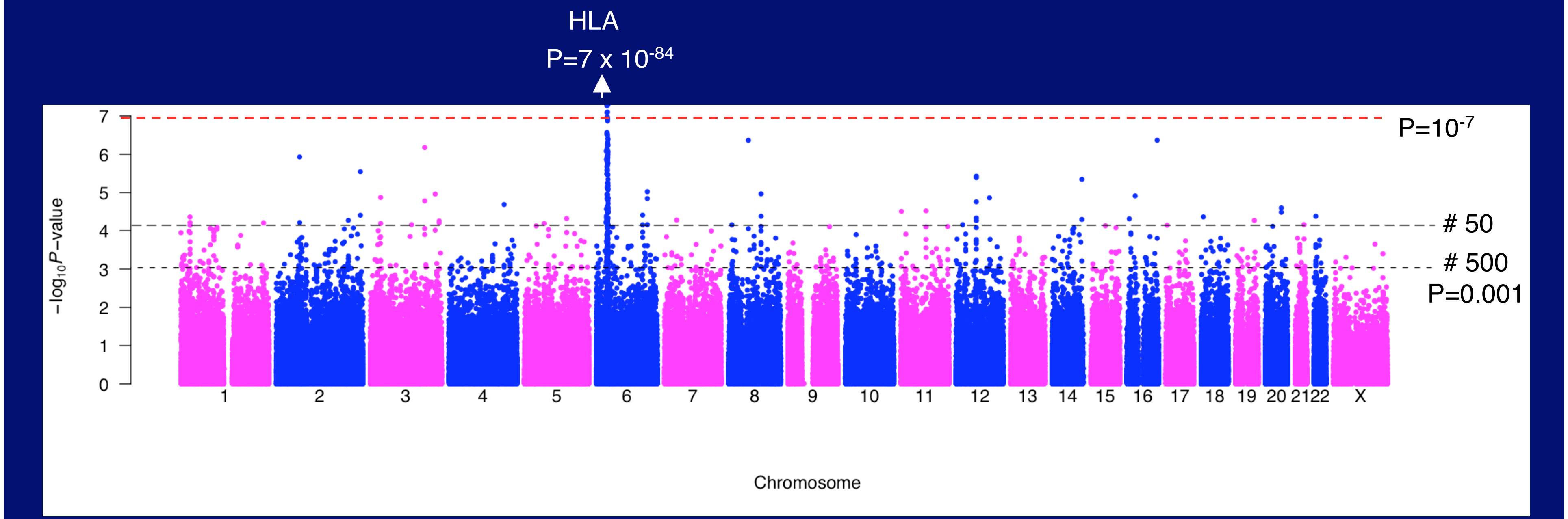
- We originally planned to use a 10% threshold, but lots of SNPs with no call rate 5-10% showed deviations from Hardy-Weinberg equilibrium



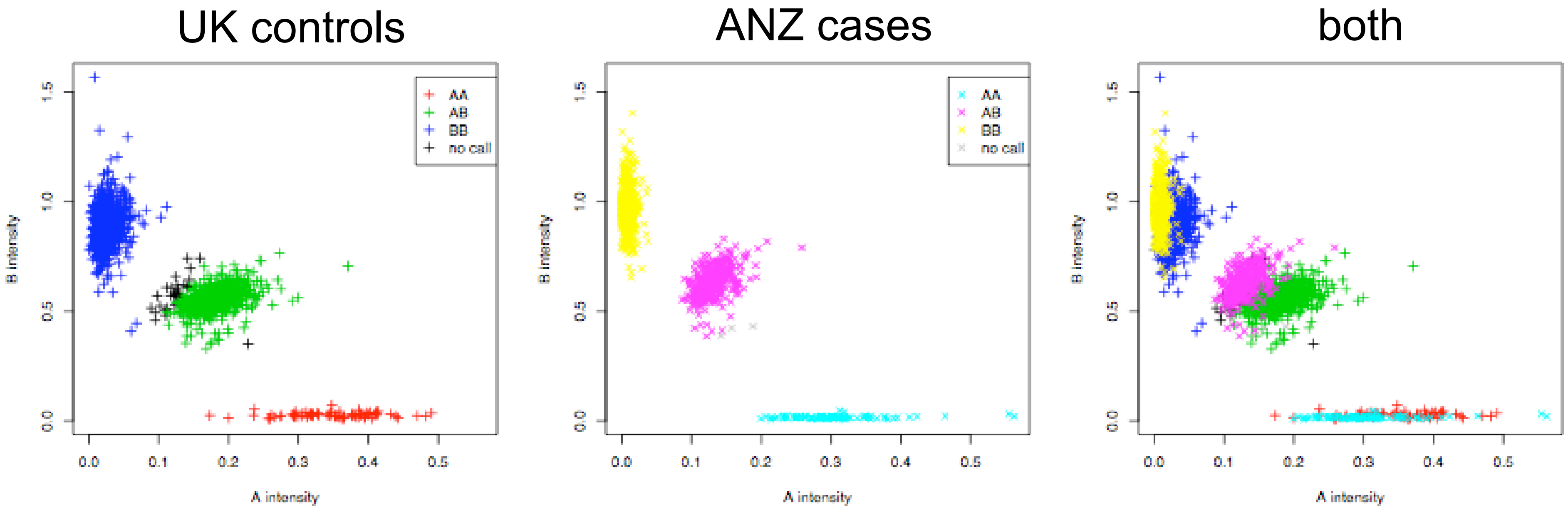
- Closer look at SNPs with call rates between 5% and 10% suggested that they were unreliable

GWAS - results

Total sample = 1618 MS cases + 3413 controls



Extra QC for associated SNPs: cluster plots



UK controls

ANZ cases

both

The replication phase

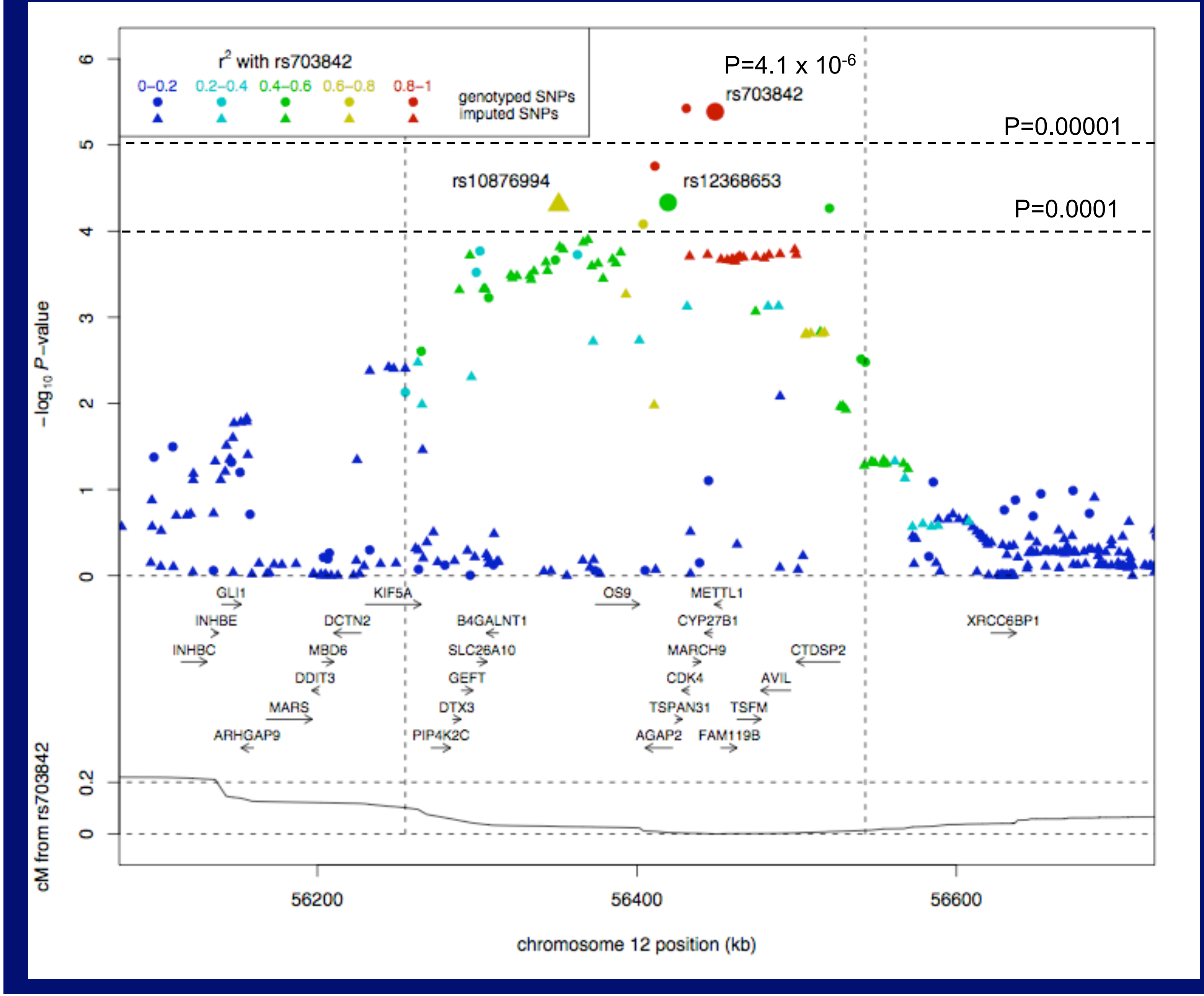
- Selected 100 SNPs for replication genotyping

- 2,256 ANZ MS cases + 2,310 ANZ controls

- Two chromosome regions on chr 12 and chr 20 showed (almost) genome-wide significant ($p<5 \times 10^{-7}$) association with MS after combining GWAS and replication data

- SNPs in 13/53 other regions with replication p-values < 0.1: more than expected by chance (p=0.002)

Chromosome 12 association: the downside of LD



rs703842

GWAS
$P = 4.1 \times 10^{-6}$

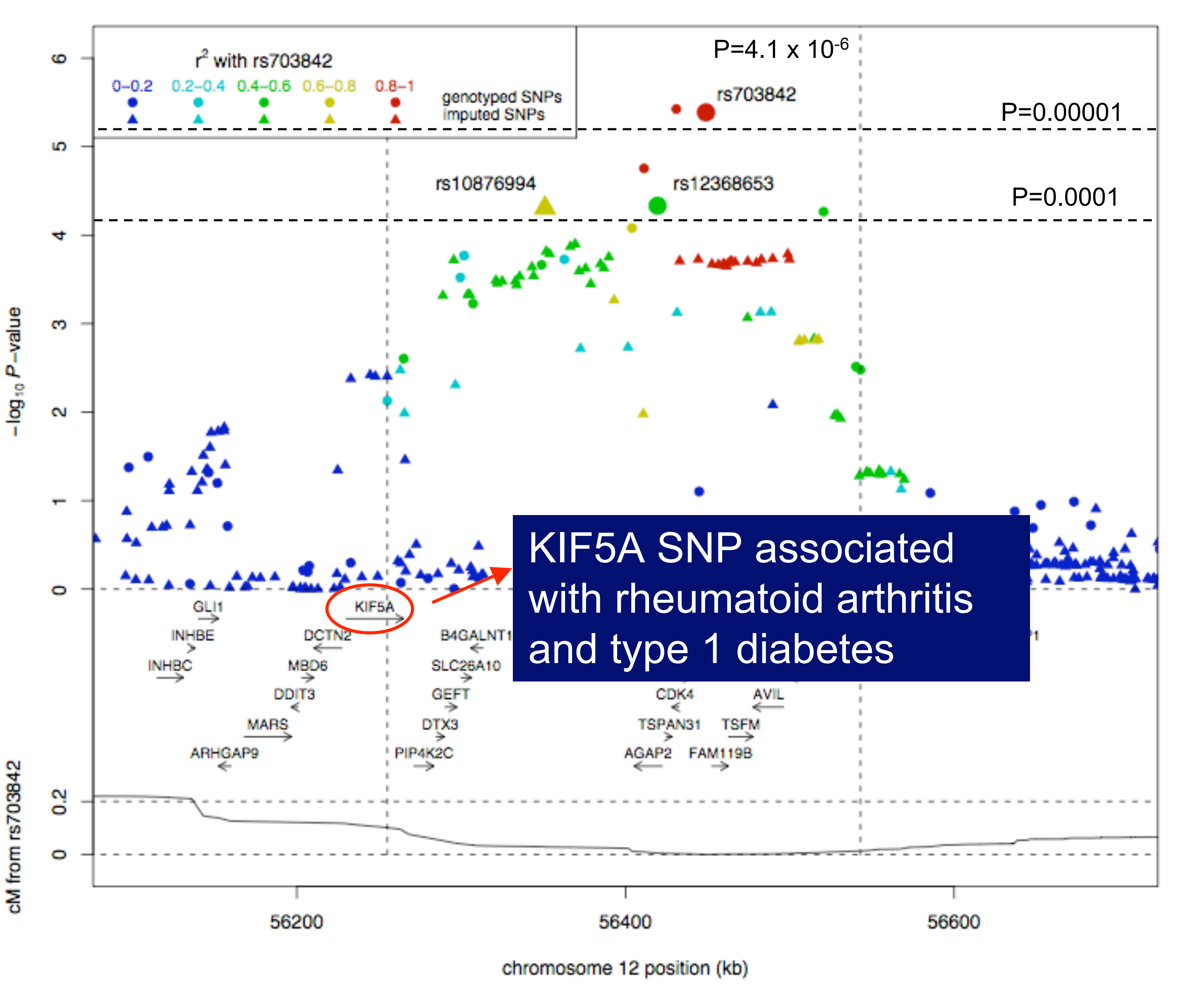
replication
$P = 1.4 \times 10^{-6}$

GWAS + rep
$P = 5.4 \times 10^{-11}$

Allele frequency 0.33

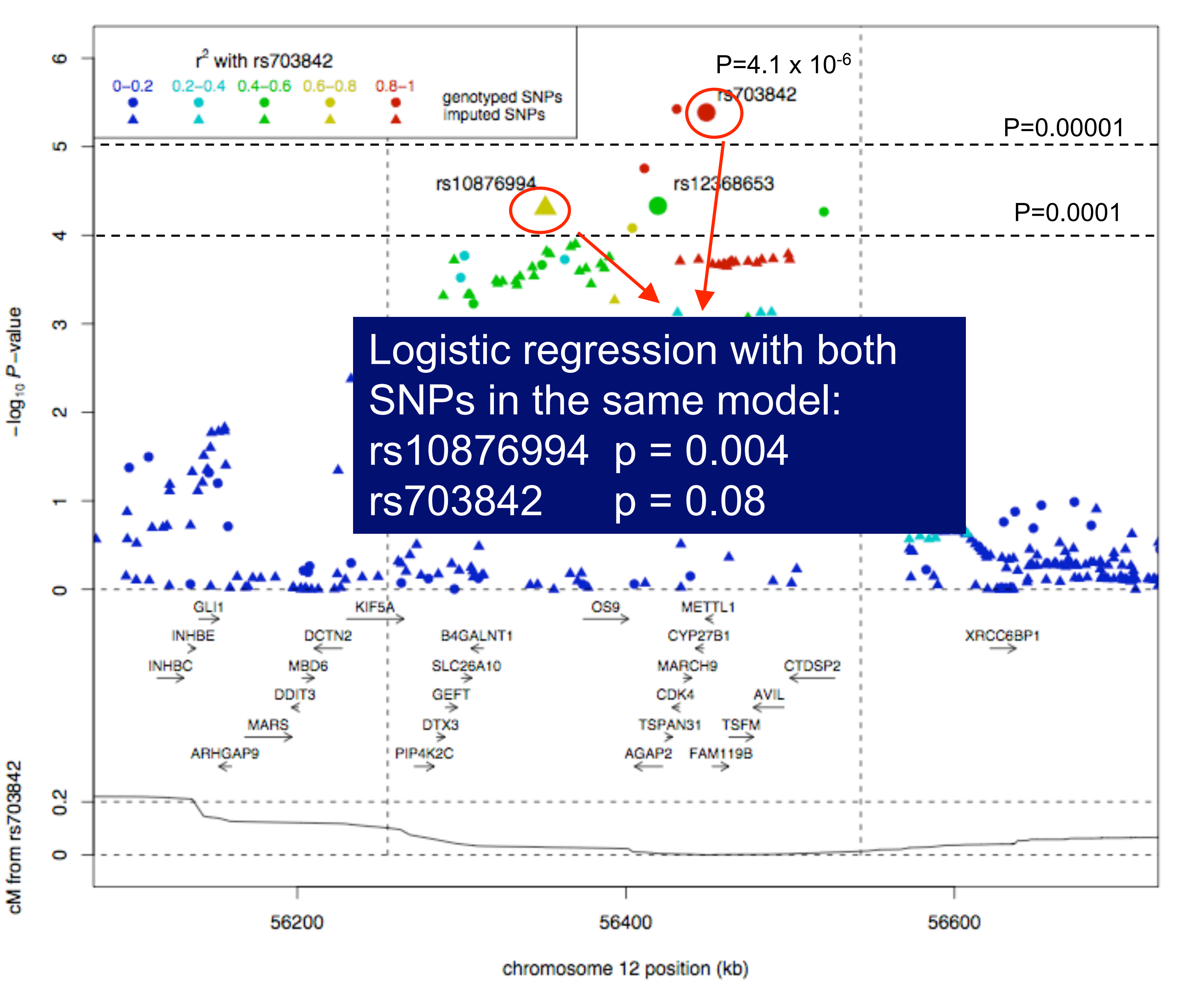
Odds ratio 0.81 (protective)

Chromosome 12 association: the downside of LD



KIF5A SNP associated with rheumatoid arthritis and type 1 diabetes

Chromosome 12 association: the downside of LD

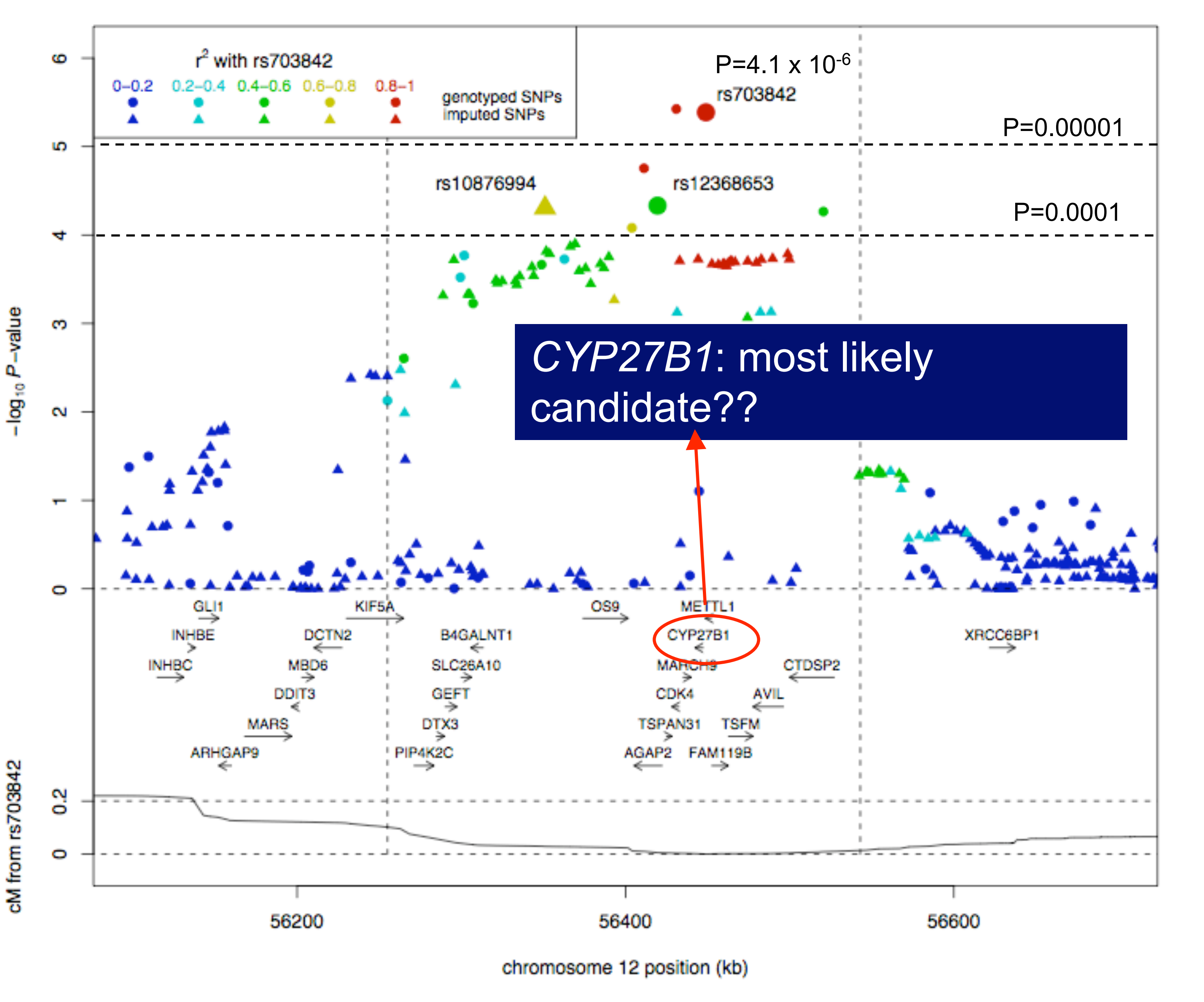


Logistic regression with both SNPs in the same model:

rs10876994 p = 0.004

rs703842 p = 0.08

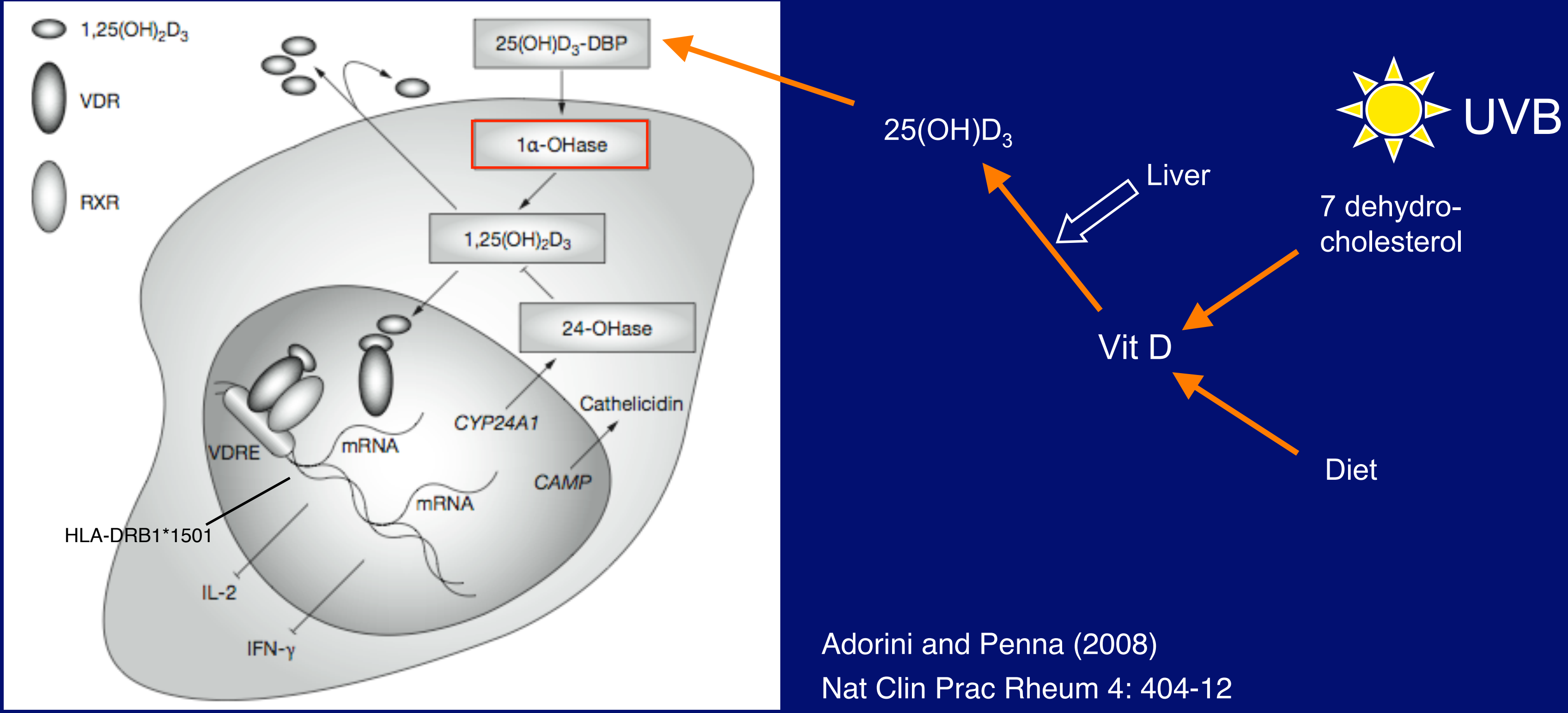
Chromosome 12 association: the downside of LD



CYP27B1: most likely candidate??

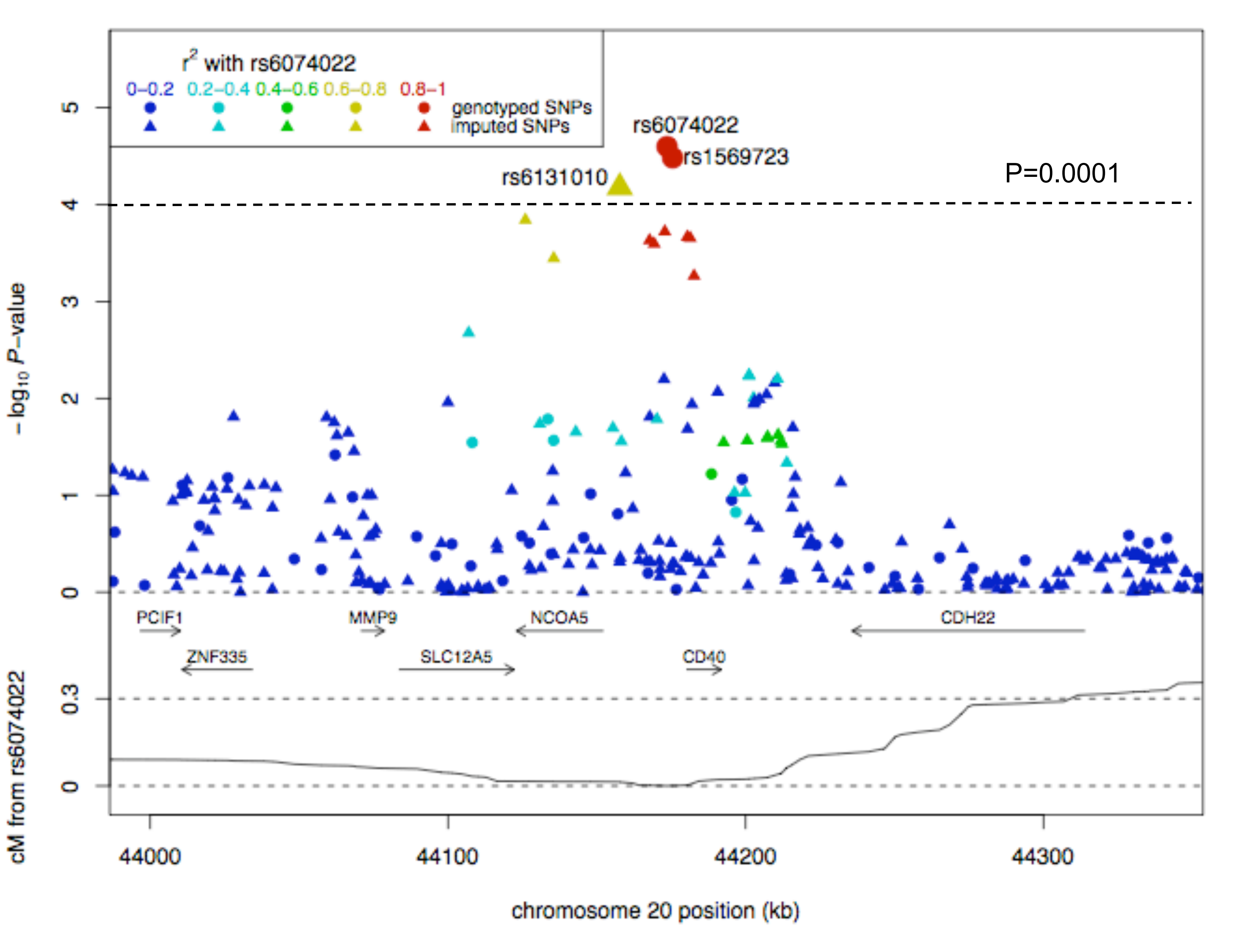
CYP27B1

- Cytochrome p450 gene family (drug metabolizing)
- Encodes 25-hydroxyvitamin D-1 alpha hydroxylase (1α–OHase)
- Converts 25(OH)D_3 to bioactive 1,25$(OH)_2D_3$
- 1,25$(OH)_2D_3$ regulates calcium metabolism and the immune system via vitamin D receptor (VDR)



Adorini and Penna (2008)
Nat Clin Prac Rheum 4: 404-12

The chromosome 20 association



rs6074022

GWAS
P = 2.5 x 10^{-5}

replication
P = 4.6 x 10^{-4}

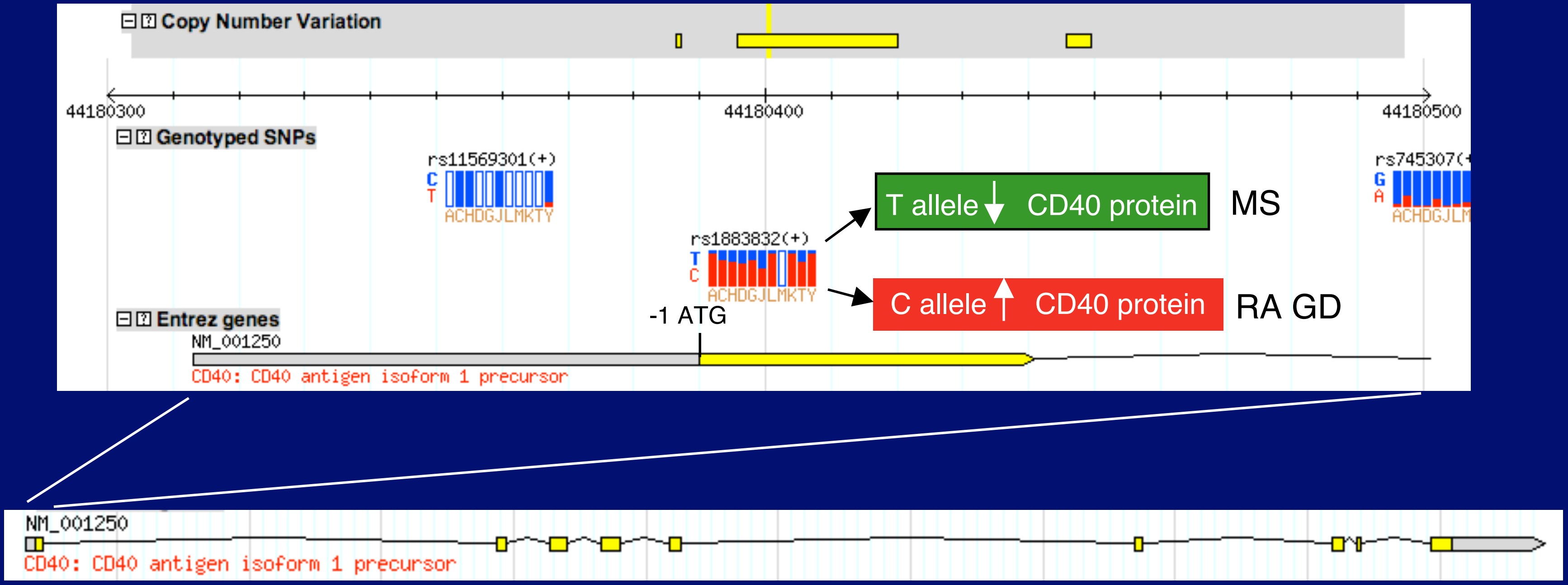
GWAS + rep
P = 1.3 x 10^{-7}

Allele
frequency 0.25

Odds ratio 1.20
(increased risk)

CD40

- Member of TNF receptor superfamily: regulates many cell- and antibody-mediated immune responses
- SNPs in CD40 are associated with risk of rheumatoid arthritis and Graves’ disease
- Functional SNP rs1883832C>T, 1 base pair upstream of the ATG translation initiation codon
- Allelic heterogeneity

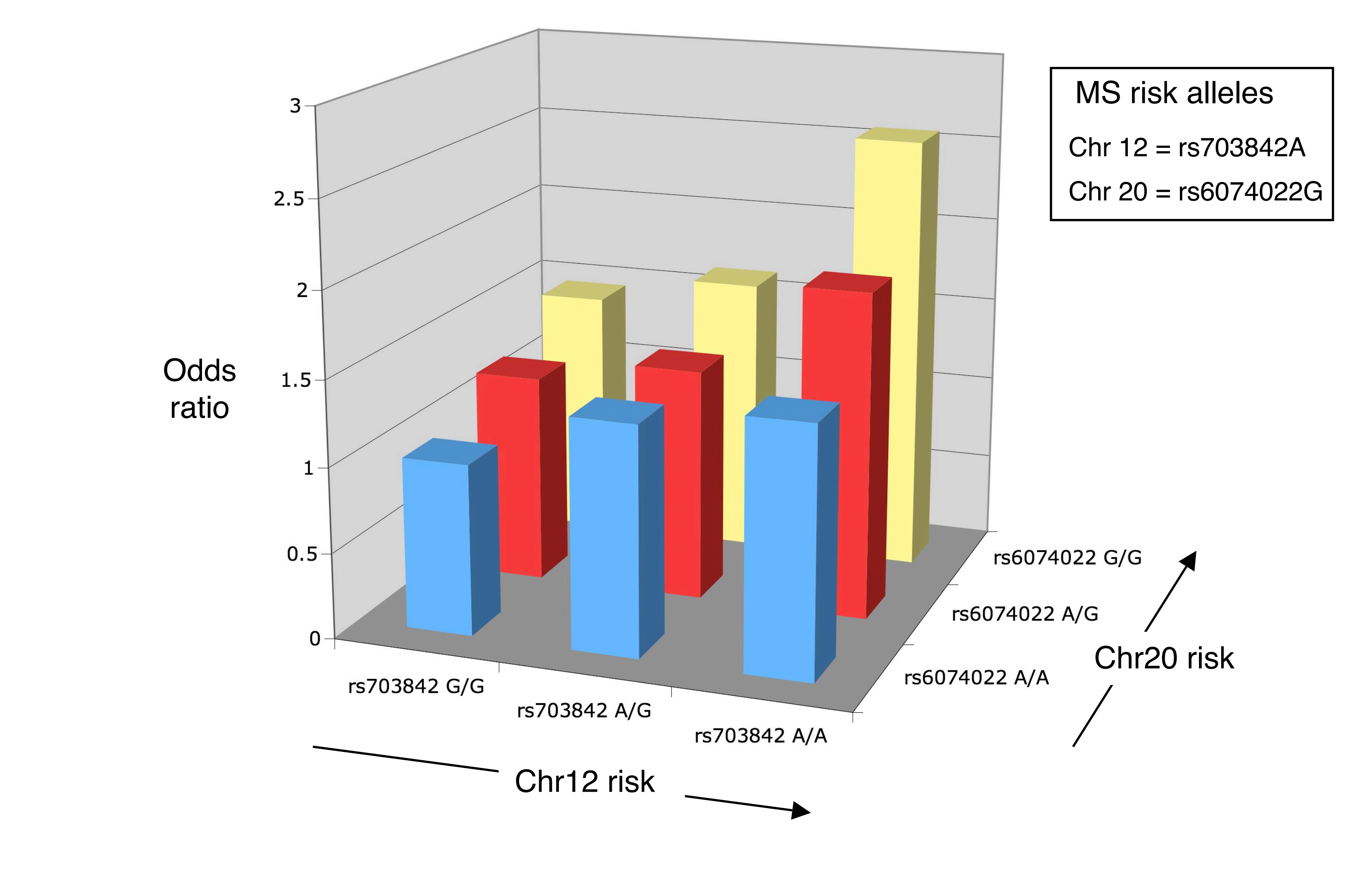


T allele ↓ CD40 protein MS

C allele ↑ CD40 protein RA GD

www.hapmap.org

Another use of logistic regression: test for gene-gene interaction



Modest evidence that each risk allele has a bigger effect in the presence of the other risk allele (p = 0.03)

Summary

- Case-control GWA studies have been very successful in the past couple of years
- Linkage disequilibrium means that most, but not all, common human genetic variation is captured by genotyping a few hundred thousand SNPs
- Small effect sizes (e.g. OR 1.2) mean that GWA studies need to be large, with thousands of cases and controls --> big collaborations
- Methods of statistical analysis are fairly straightforward, but care is required to clean data
- The ultimate test of any association: replication in an independent population

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