



# The genetics of MS

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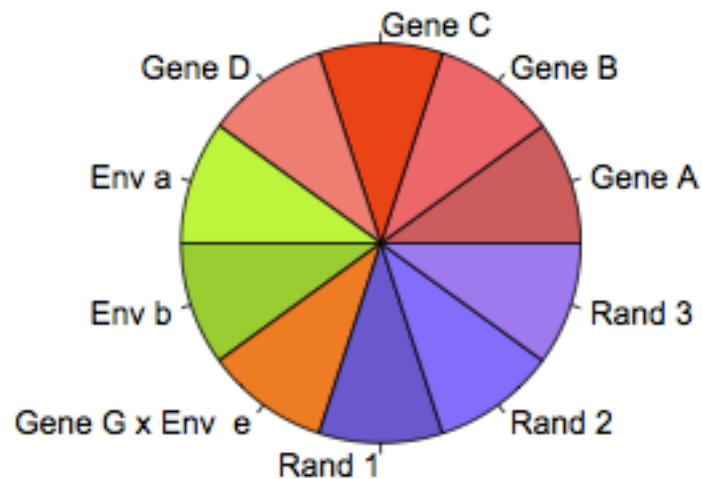
  
research thanks to you

 **UTAS**  An institute of the  
University of Tasmania

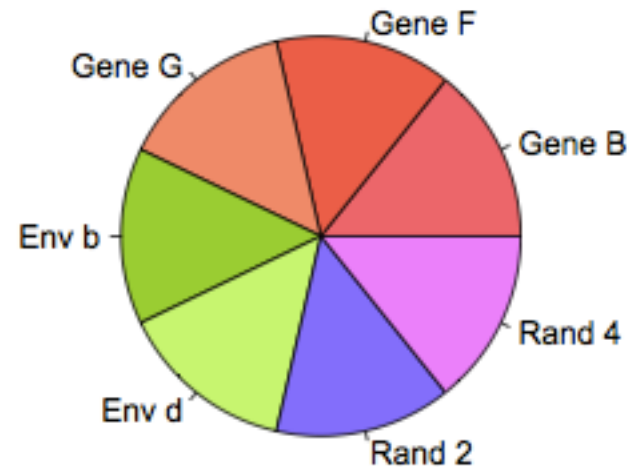
# MS is genetically complex

- (As opposed to **single gene** disorders like Huntington's disease or cystic fibrosis)
- There are many genetic risk factors (100s or 1000s?), exerting subtle effects, interacting with each other and with environmental risk factors
- This makes the genetic risk factors and environmental risk factors harder to find

*Epidemiologist Ken Rothman: **causal pies***



Causal pie for one person with MS



Causal pie for another person with MS

# Why is it useful to find genetic risk factors for MS?

- Find an environmental risk factor (e.g. low vitamin D)  
→ possible to modify behaviour
- Can't change the genes you inherit

# Why is it useful to find genetic risk factors for MS?

- 1 Provide new insights into the pathology of MS, particularly what triggers MS

*Which is more important in the onset of MS:  
neurodegeneration or inflammation?*

- Directions for further research
- Change actions of particular genes  
(change how easily they are turned on, or how much protein they make when they are turned on)  
→ treatments?

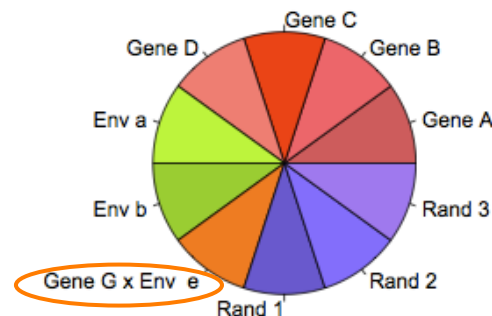
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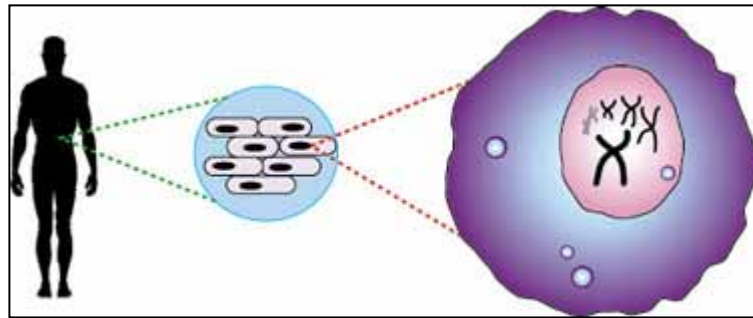
- 2 Gene-environment interaction. Identify people who are more genetically susceptible to the effects of certain environmental risk factors



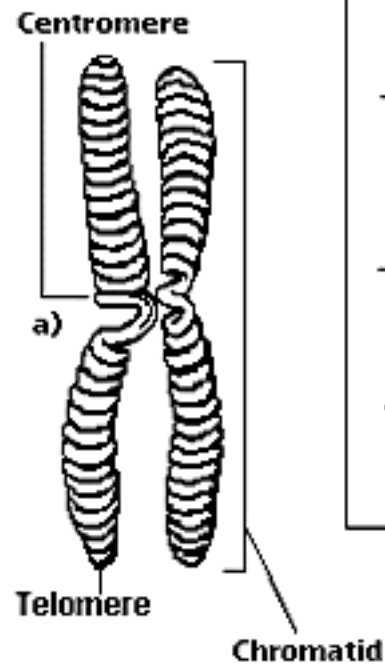
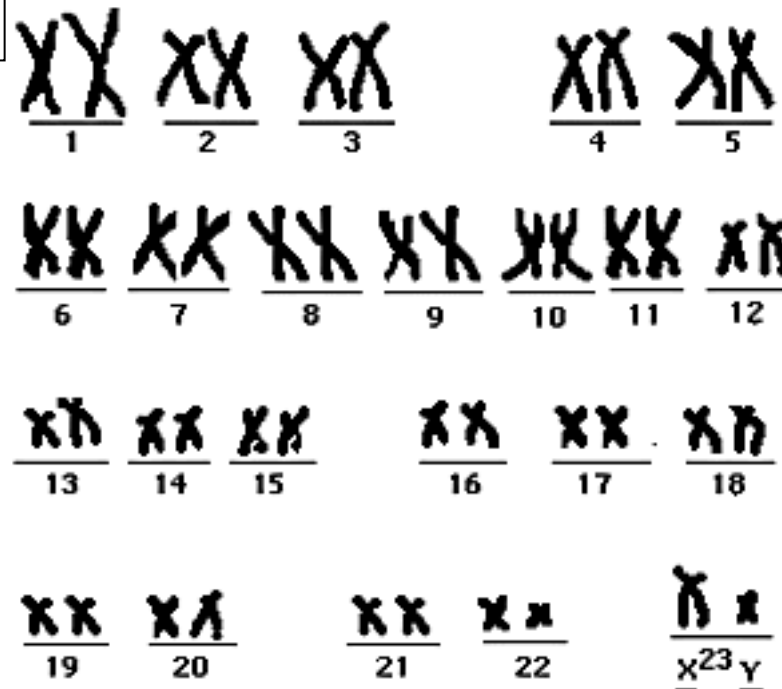
[for a very complex disease like MS, this sort of prediction is *never* going to be perfect]

DNA, genes, and genetic risk factors

# Every cell has DNA bundled into chromosomes

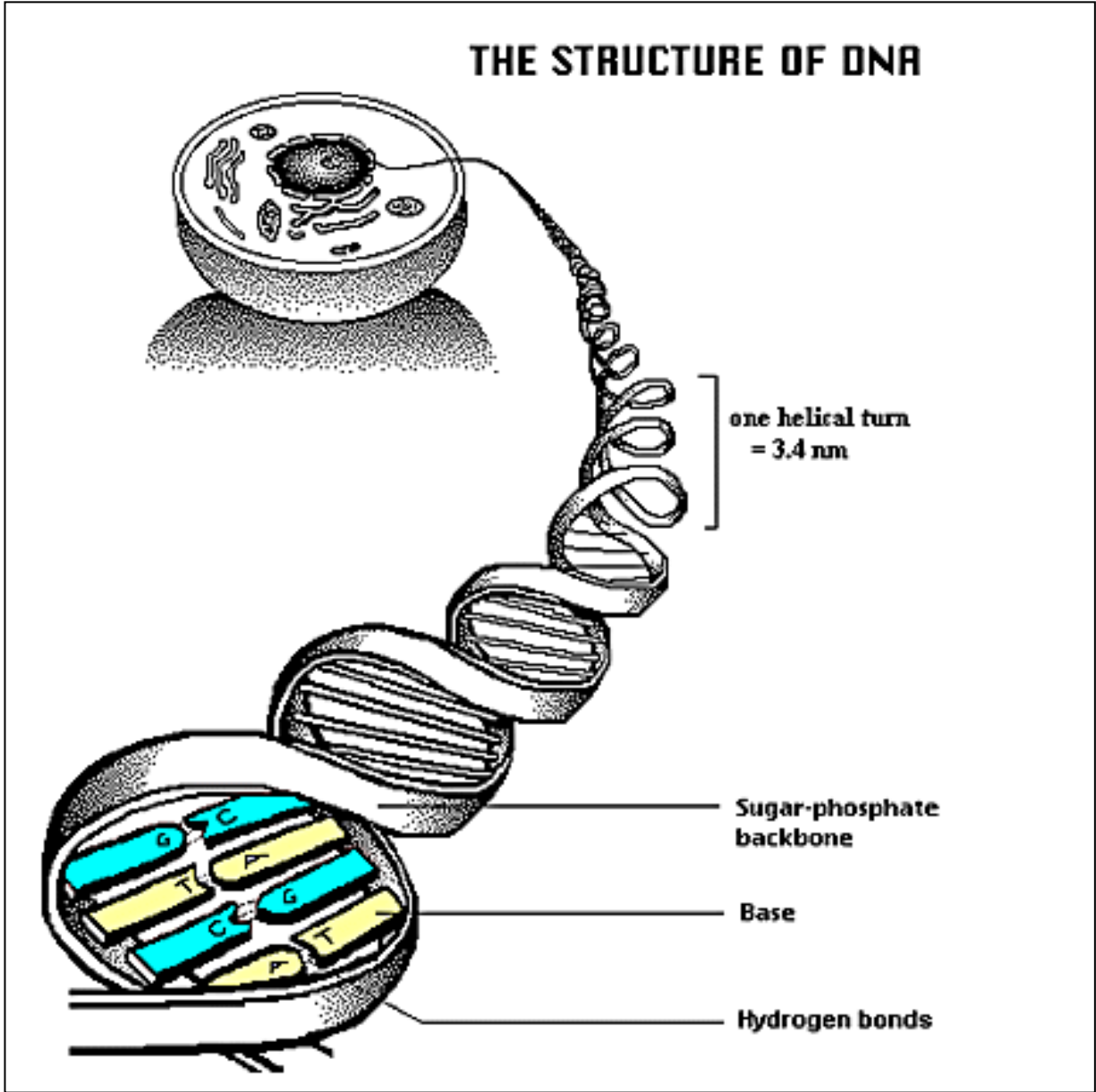


## HUMAN CHROMOSOMES



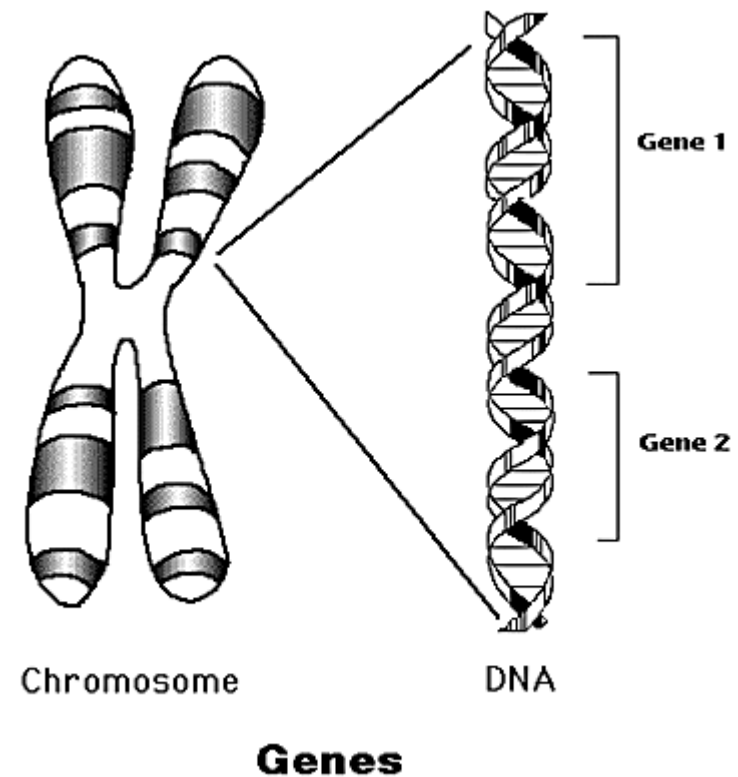
c)

# THE STRUCTURE OF DNA

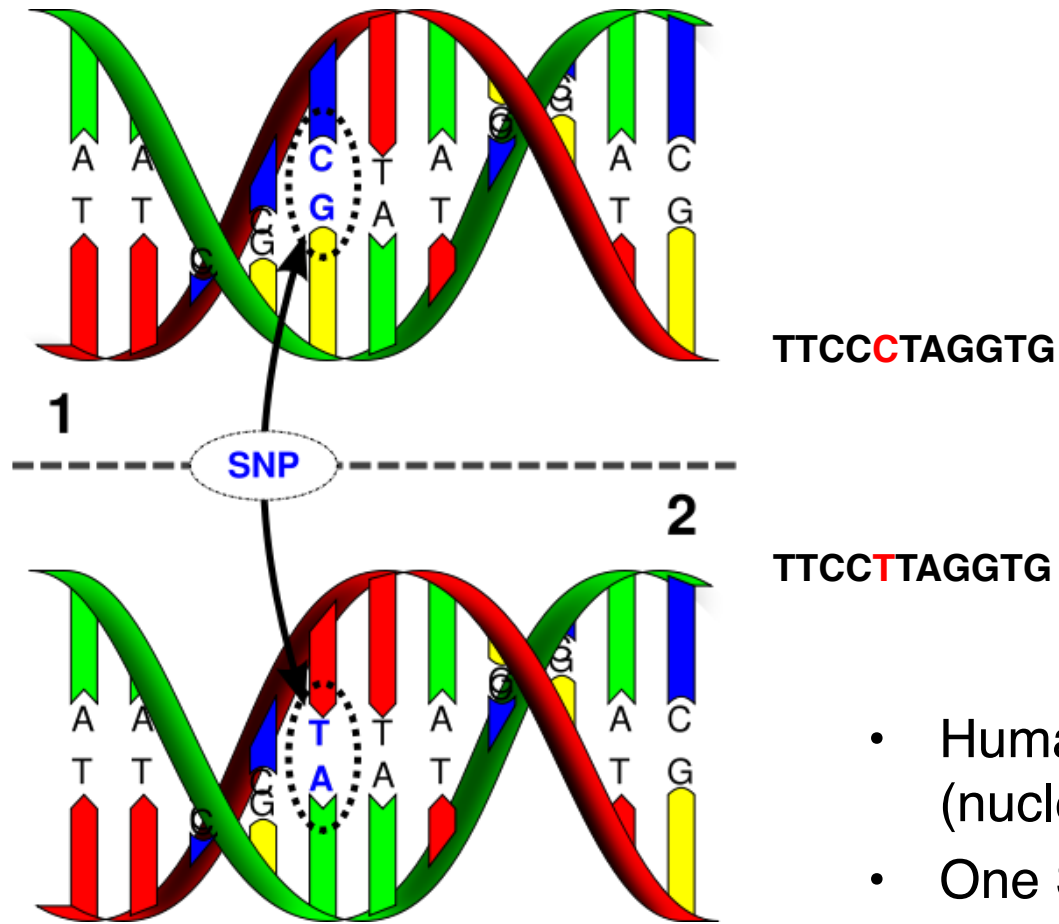


# Genes

- Around 25,000 different genes along the human DNA sequence
- Determine cell type and function
- Make the **proteins** that make us
- Control processes



# The most common type of genetic variant: single nucleotide polymorphisms (SNPs)



- Human DNA sequence: 3 billion letters (nucleotides)
- One SNP every 300 letters
- Around 10 million SNPs

## Genetic risk factors for MS

TTCC<sup>C</sup><sub>T</sub> TAGGTG

	<b>people with MS</b>	<b>Controls</b>
<b>C</b>	240 (12%)	200 (10%)
<b>T</b>	1760 (88%)	1800 (90%)
<b>Total</b>	<b>2000</b>	<b>2000</b>

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P-value = 0.05

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genetic **association studies**

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genetic **association studies**

## Which genetic variants should you test?

- Strategy until recently
  - **Candidate gene association studies**: guess which of the 25,000 genes might influence disease
  - Major discovery in 1972: one variant of the gene *HLA-DRB1* is associated with increased risk of MS  
(this variant carried by around 30% of Tasmanians overall, but by approximately 60% of Tasmanians with MS, odds ratio 3.5)
  - In the years after the whole human genome was sequenced in 2000, millions of genetic variants were discovered
  - Variants in many genes tested for association with MS, but little success between 2000 and 2006  
(bad guesses, and studies too small to detect subtle effects)

# HLA-DRB1 plays an important role in the immune system



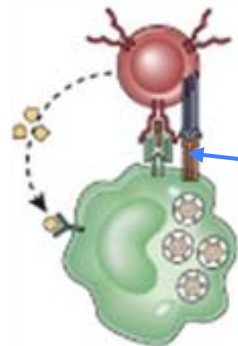
Phagocytosed microbes in macrophage

Macrophages: immune cells that chop up microbes (phagocytosis)



Helper T lymphocyte

Helper T cells: immune cells that activate other types of immune cells



HLA-DRB1 makes a protein which sits on the surface of macrophages and holds bits of chopped up microbe to be recognized by helper T cells

Cells (T lymphocytes)

**Activate macrophages to kill phagocytosed microbes**

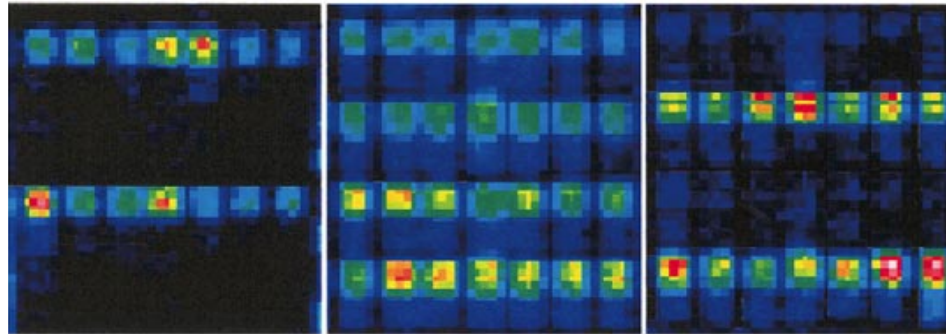
Different variants of HLA-DRB1 influence helper T cell recognition, and consequently how helper T cells activate the immune system



Big technological breakthrough in 2007: “gene chips” to measure hundreds of thousands of SNPs in one person’s DNA



TTCC<sup>C</sup>TAGGTG  
          <sup>T</sup>



C/C

C/T

T/T

Kennedy et al. (2003)  
Nat Biotech 21: 1233

Now no gene guessing is required!

Can test all 25,000 genes: **genome-wide association study**

## First genome-wide association study for MS in 2007



The NEW ENGLAND  
JOURNAL of MEDICINE

### Risk Alleles for Multiple Sclerosis Identified by a Genomewide Study

The International Multiple Sclerosis Genetics Consortium\*

N Engl J Med 2007;357.

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- 931 patients with MS from US and UK, using their parents as controls
- Discovered associations with two more immune system genes
  - Interleukin 7 receptor (IL7R), odds ratio 1.18
  - Interleukin 2 receptor alpha (IL2RA), odds ratio 1.19



- The Australia and New Zealand MS Genetics Consortium
- 11 institutions
- 40+ researchers
- 4,500 people with MS
- \$1.5M MS Research Australia / ARC

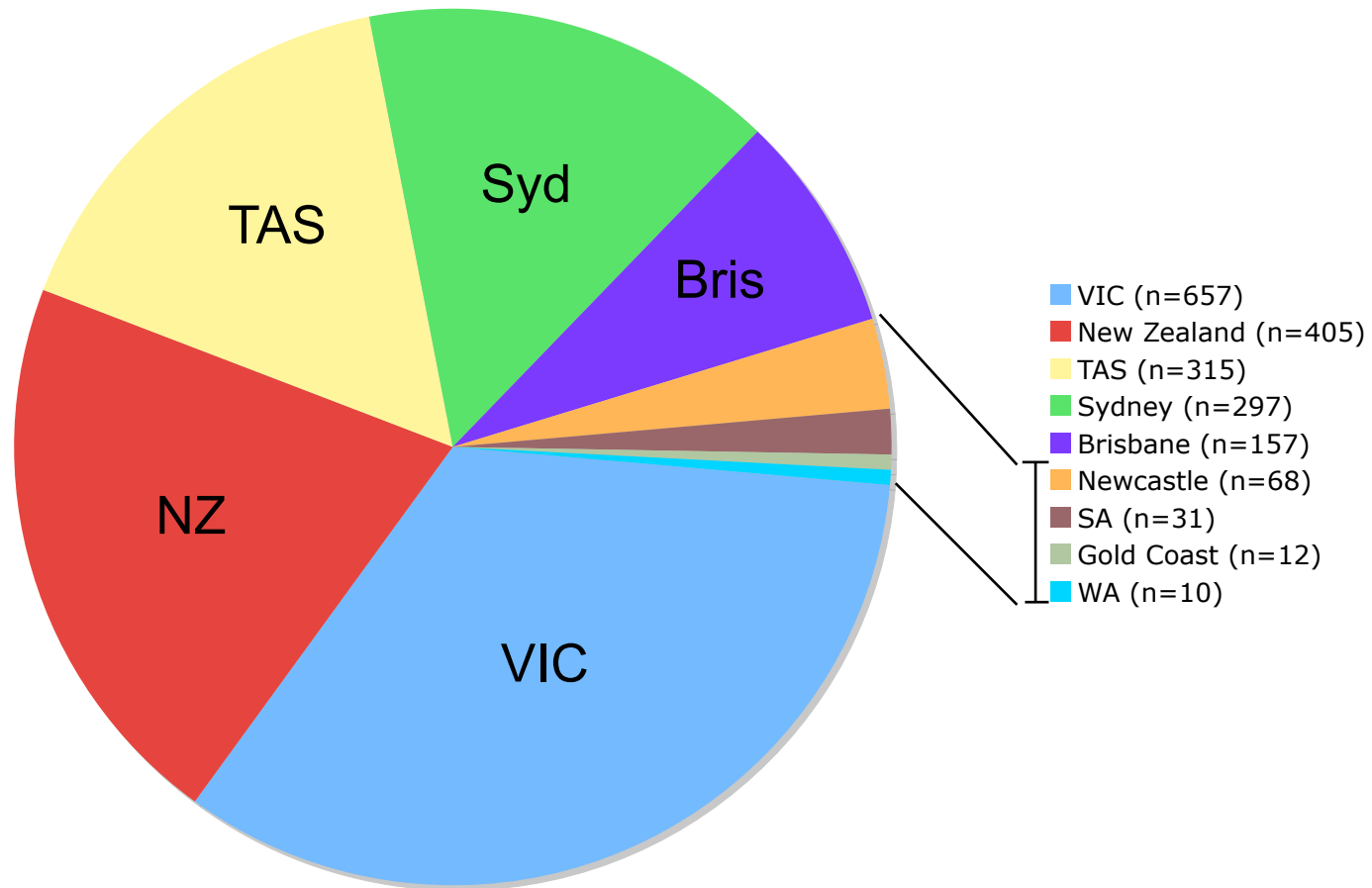


## Australian and New Zealand MS genetic case-control study

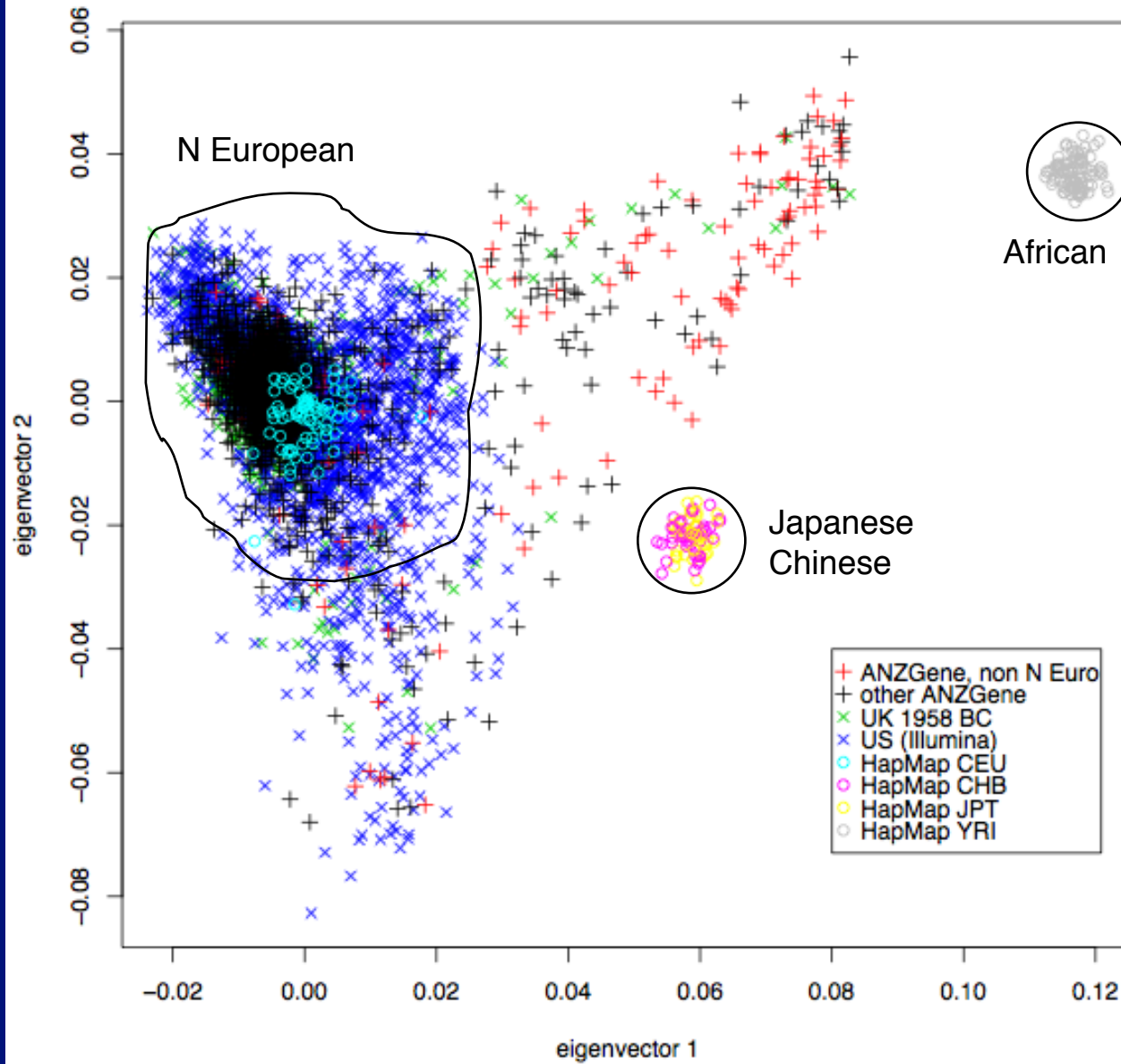
- Phase 1
  - Measure ~300,000 genetic variants (SNPs) in 1618 Australian & New Zealand MS cases using gene chips
  - Compare with 3413 controls from the UK and US (public data)
- Phase 2
  - Select the most promising 100 variants from Phase 1
  - Try to replicate results for these variants in 2256 other cases and 2310 controls from Australia and New Zealand

# Origins of cases in phase 1

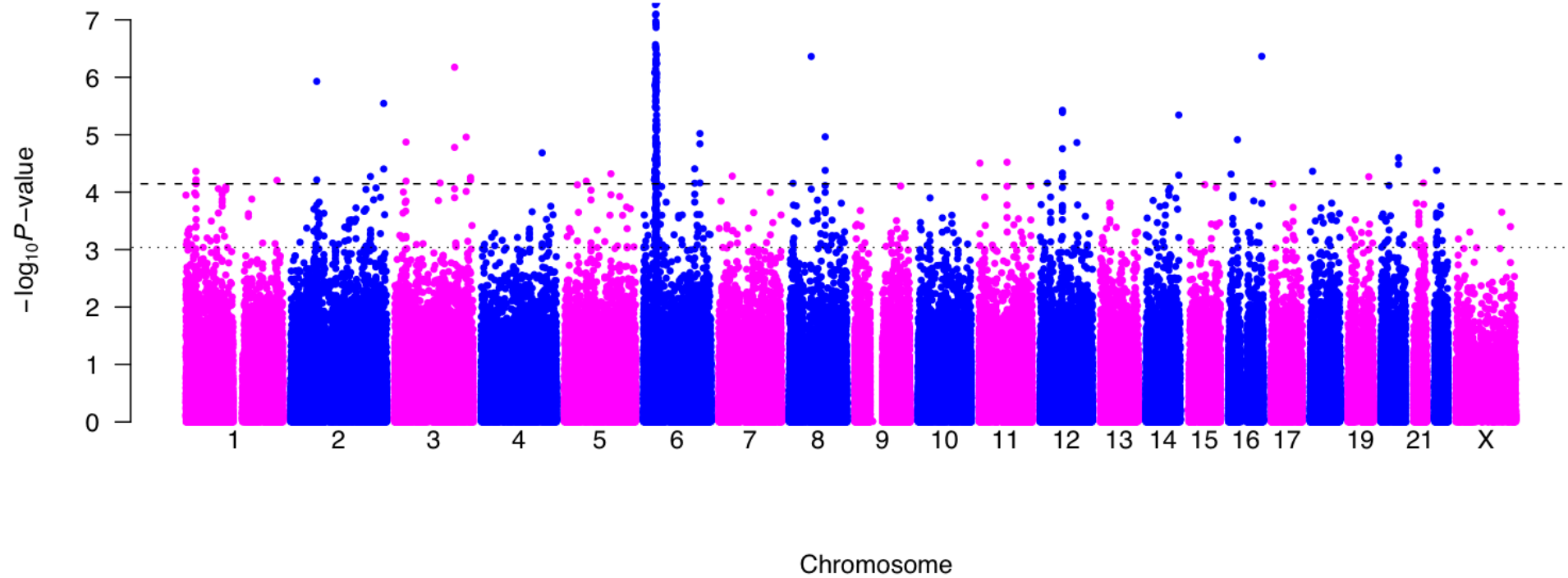
ANZgene GWAS - MS samples by origin



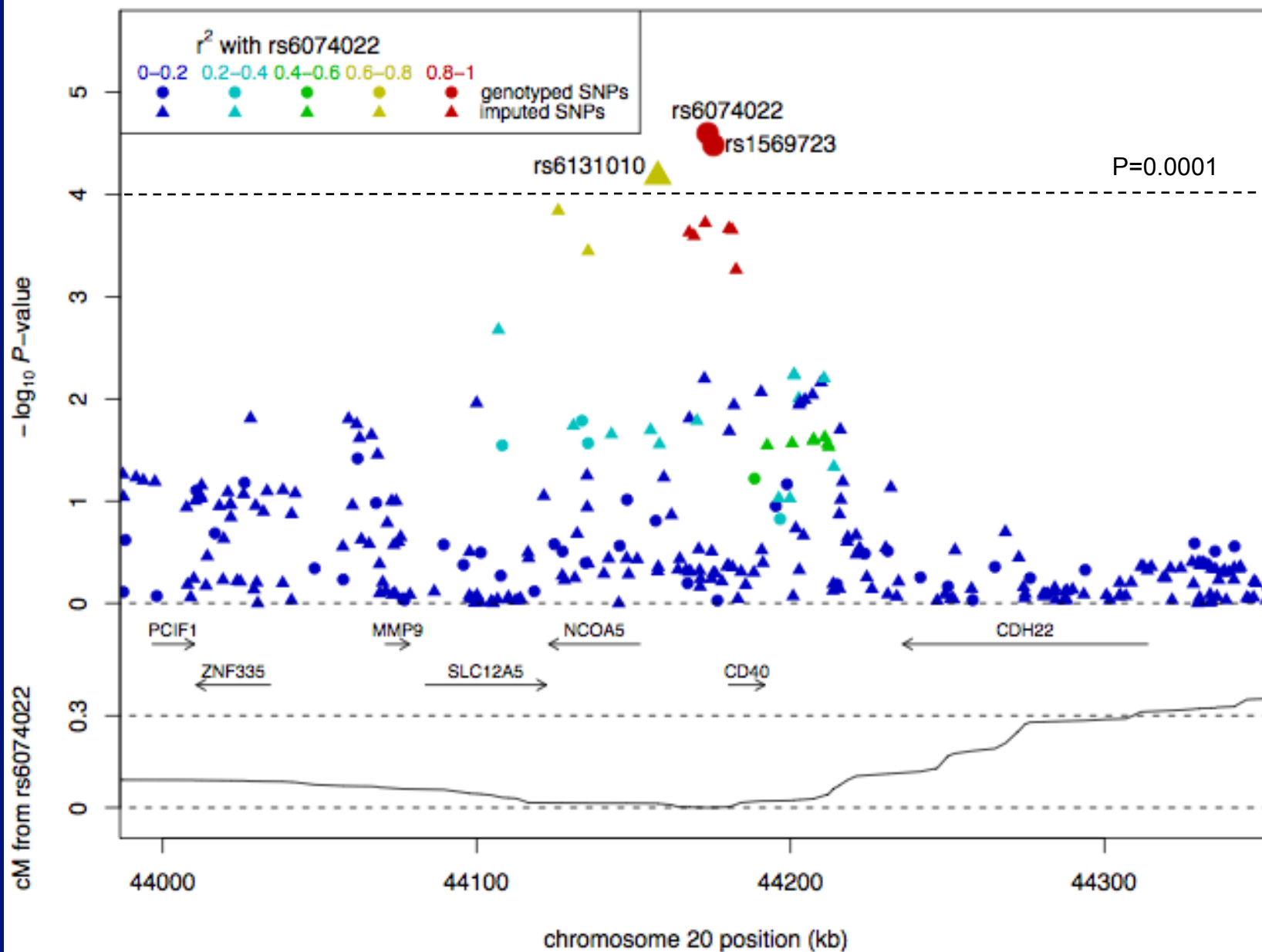
# Quality control – ethnic ancestry



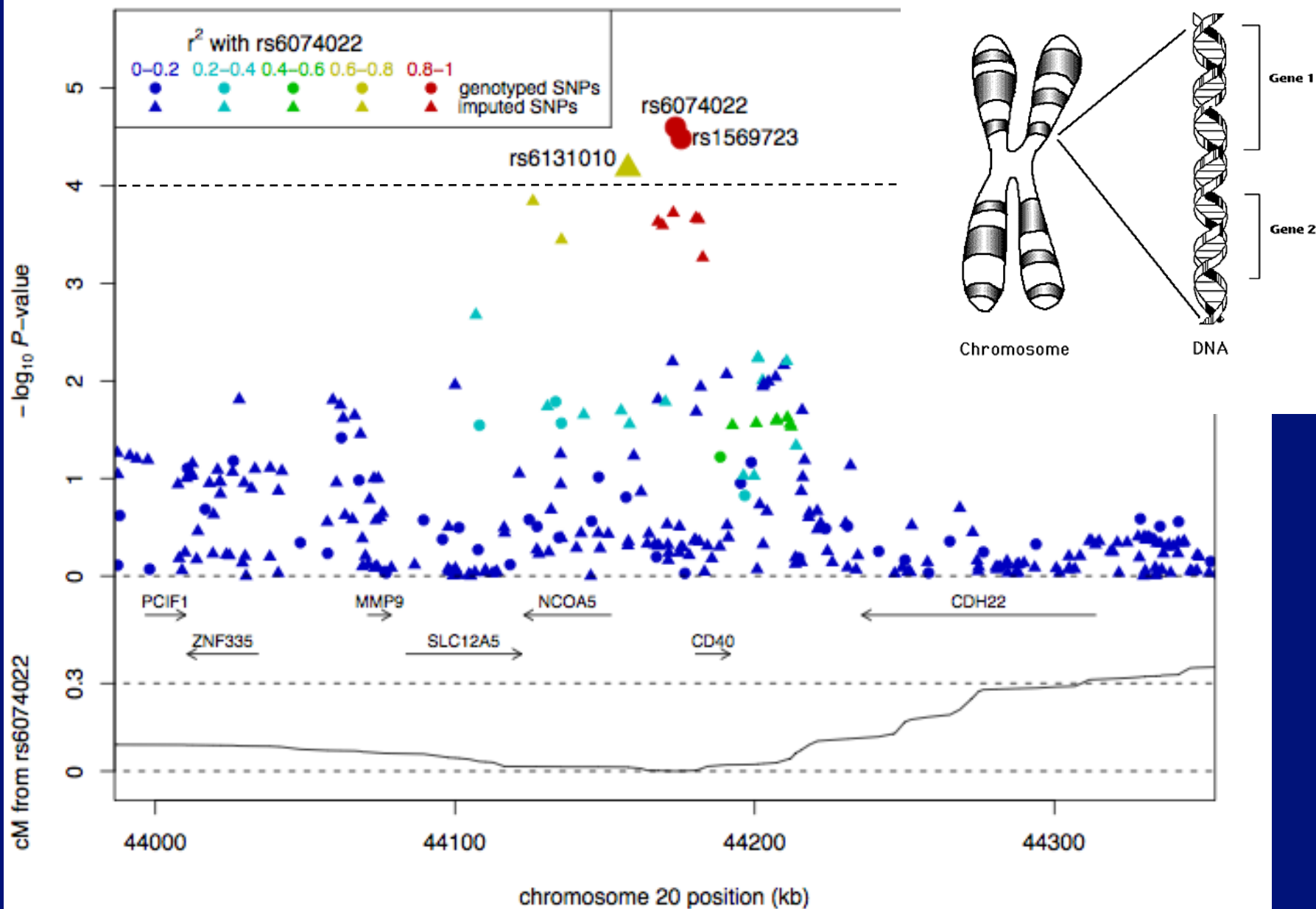
# Phase 1: testing 300,000 variants



# Phase 2: results in two chromosome regions replicated



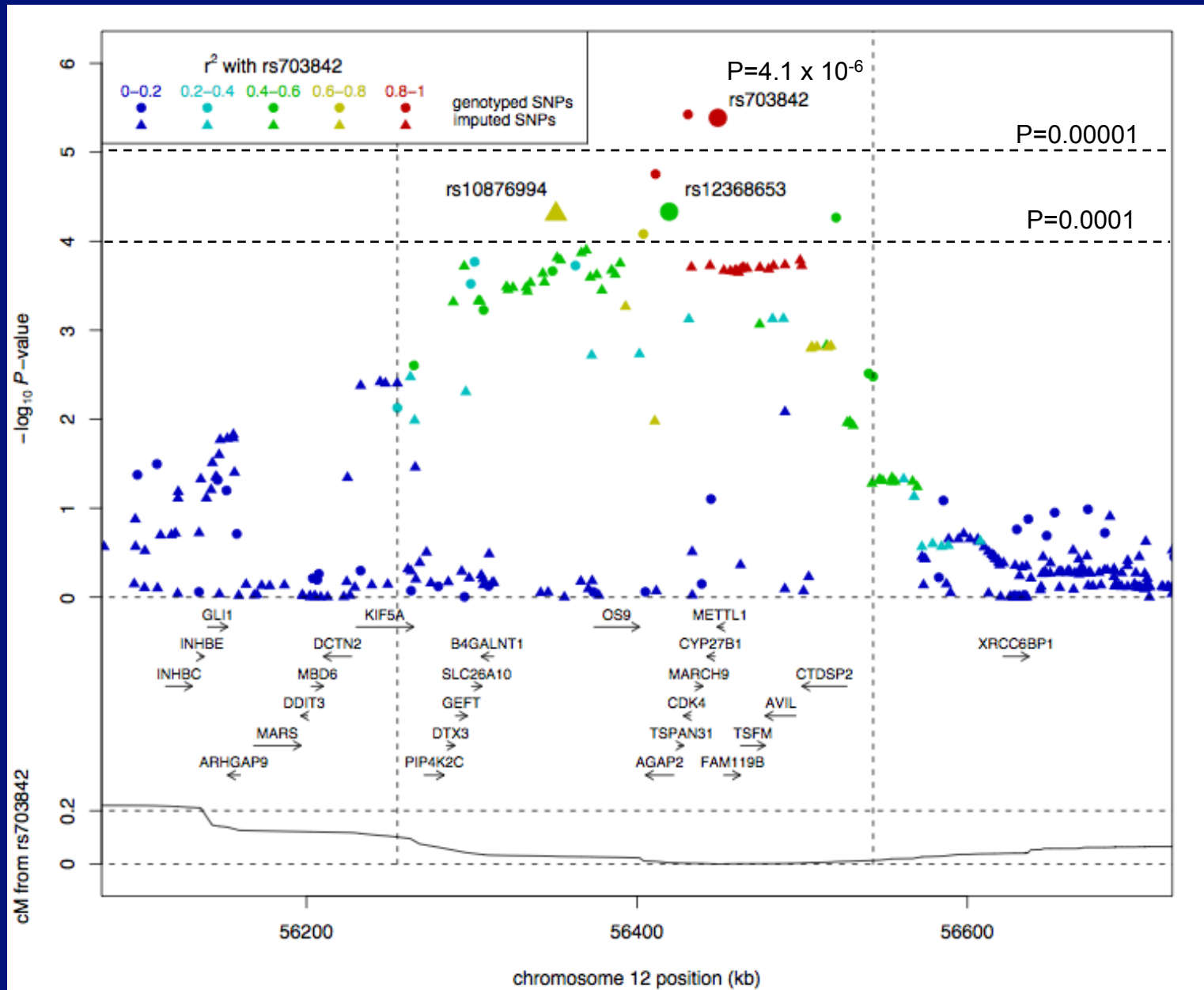
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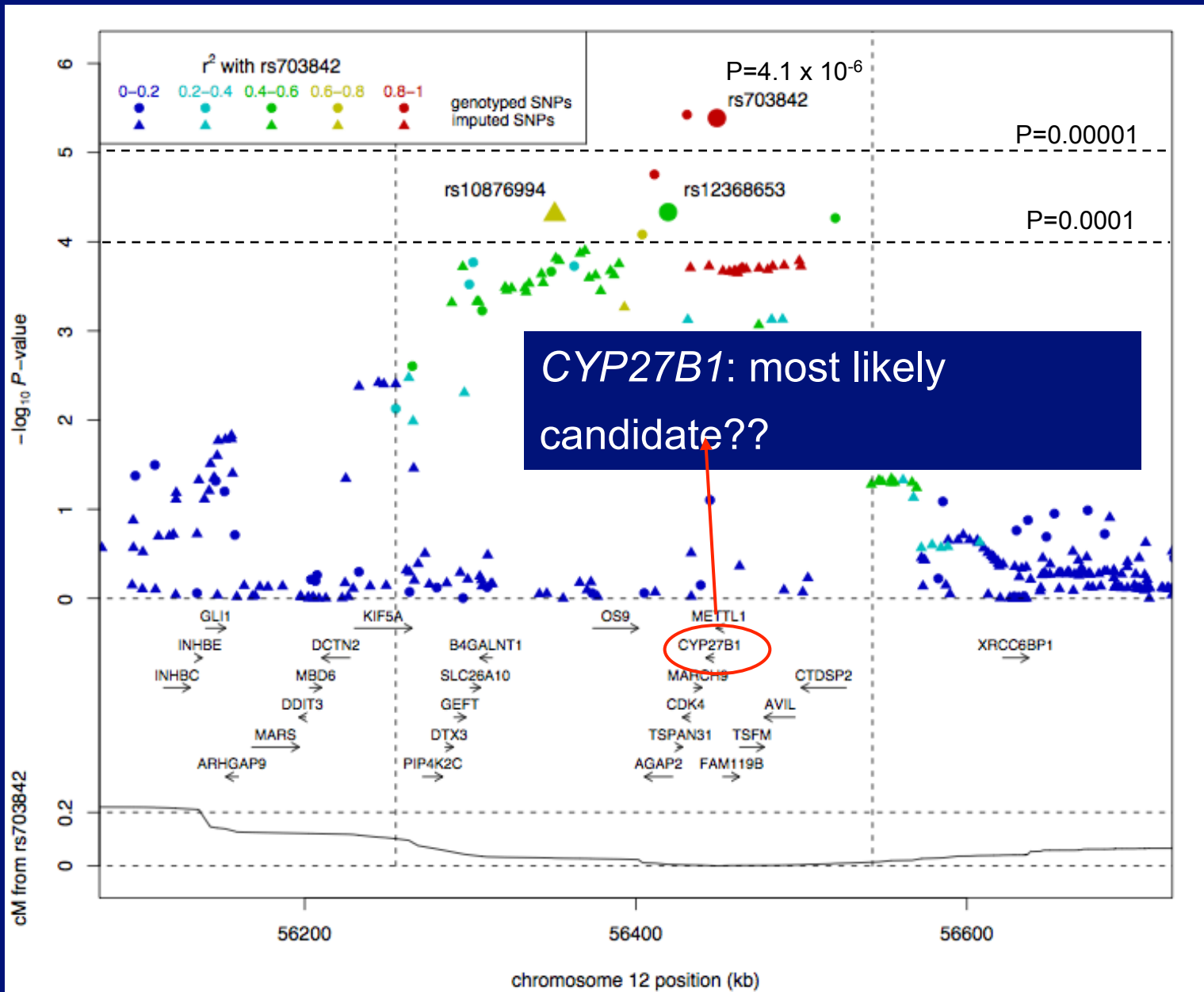
# CD40

- Codes for a protein which is a signalling molecule
- This signalling protein plays a broad role in a range of immune responses
  - Cell-mediated responses (e.g. T helper cells)
  - Antibody-mediated responses
- The genetic variant which increases risk of MS reduces the amount of protein that is made
- This same variant decreases risk of other autoimmune diseases:
  - Rheumatoid arthritis
  - Autoimmune thyroid disease

# Other region: not so easy to tell which is the causative gene



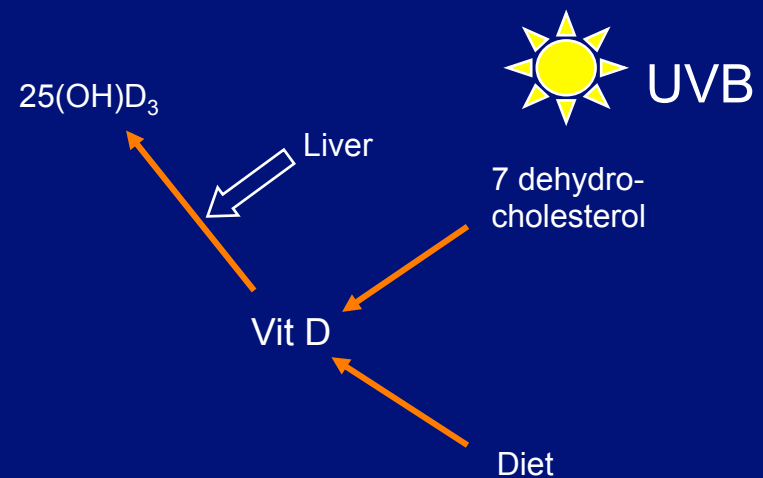
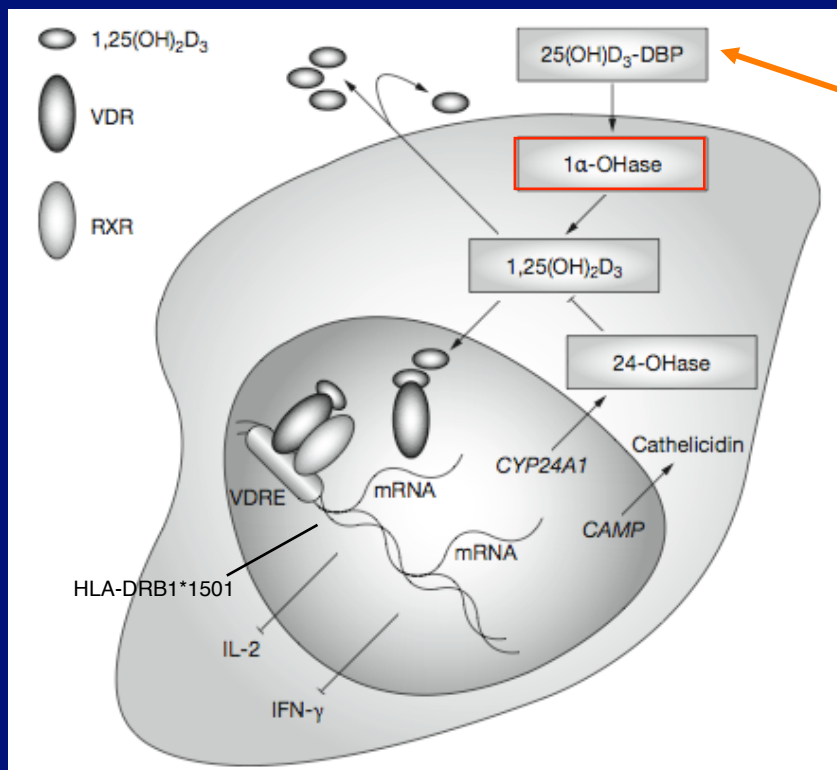
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# CYP27B1 - function

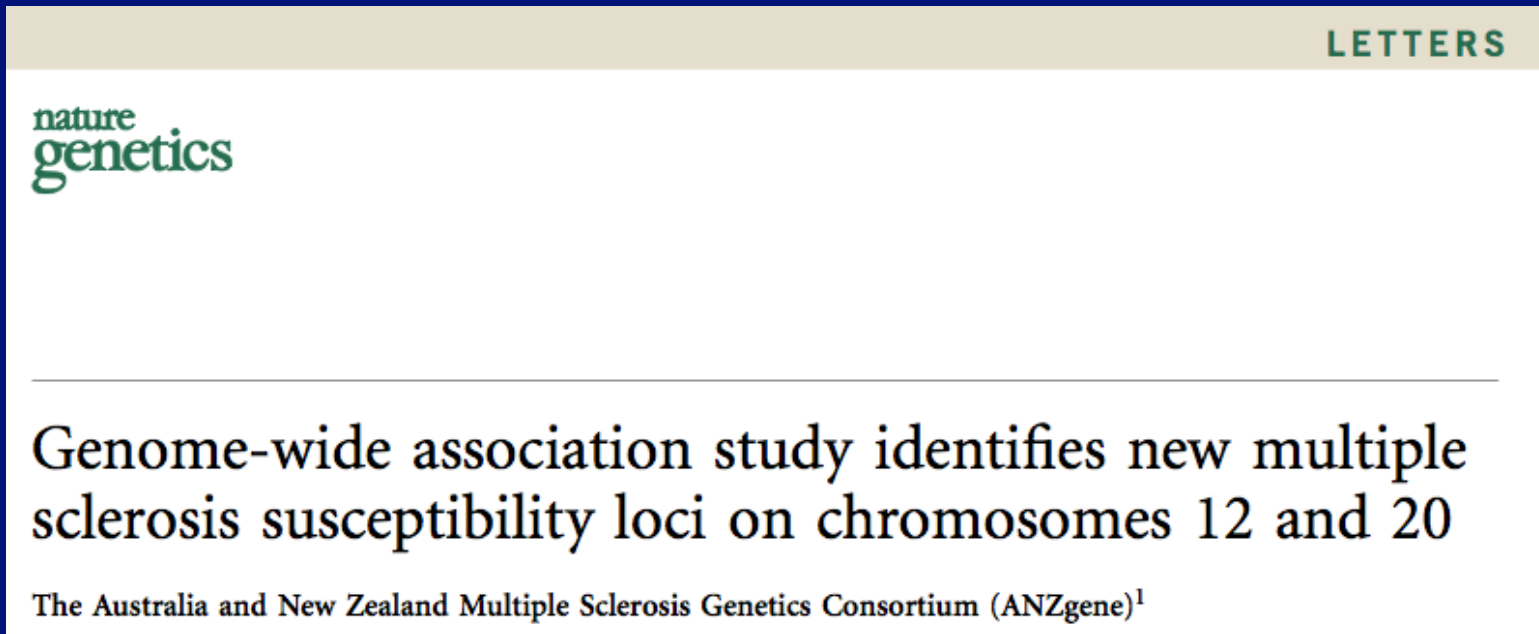
- Codes for an protein that converts vitamin D from a stored form to an active form
- The active form of vitamin D helps regulate the immune system

**Hypothesis:** Dysfunction of *CYP27B1* and lower levels of active vitamin D create an inflammatory environment conducive to autoimmunity



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

# Conclusions from our study (published 2009)



- Further evidence for genetic overlap between organ-specific autoimmune diseases
- Identified a possible genetic link between vitamin D metabolism and risk of MS - *can modulation of this pathway be used to treat MS, and other autoimmune diseases?*
- CD40 signalling is important in MS: working in the opposite direction compared to other autoimmune diseases

# Conclusions from another genome-wide association study published in 2009

De Jager et al, Nature Genetics: discovered associations with 3 more immune genes

“Thus, we inform the ongoing debate of the relative roles of neurodegeneration and inflammation in the onset of MS by reporting a preponderance of current genetic evidence in favour of early immune dysregulation that may trigger secondary neurodegenerative processes.

A definitive evaluation of this question awaits a more complete map of genetic susceptibility factors and a more comprehensive understanding of the functions of the associated genes in different cell types.”

## “a more complete map of genetic susceptibility factors”

- Now, 27 genes confirmed to be associated with MS
  - HLA-DRB1, IL7R, IL2RA, CYP27B1, CD40, HLA-A, HLA-DPB1, MMEL1, EVI5, CD58, RGS1, KIF21B, CBLB, TMEM39A, IL12A, PTGER4, OLIG3, IL7, ZMIZ1, CD6, TNFRSF1A, MPHOSPH9, CLEC16A, IRF8, STAT3, CDC37, TYK2
  - Most, but not all, of these genes are involved in the immune system
  - All variants have subtle effects (increase risk of disease by less than 20%) apart from HLA-DRB1
- New study looking at DNA from nearly 10,000 people with MS, including 800 people from Australia and New Zealand:
  - evidence of another 24 genes that are associated with MS, bringing the total number to 51
- These discoveries still don't explain all of the familial clustering of MS, so there are more susceptibility factors to be found
- But a lot of progress in the last 4 years

“a more comprehensive understanding of the functions of the associated genes in different cell types”

- Study underway to do this (collaboration with Helmut Butzkueven, University of Melbourne)
  - Collect blood samples from people with MS and people without MS of the same age and sex
  - Separate various types of immune cells from the blood on the same day that it is collected (this takes all day for two samples)
  - Measure how much protein the genes make in various cell types, and see whether this is correlated with the DNA variants that are associated with MS (just as the **CD40** variant associated with MS reduces the amount of CD40 protein that is made)
  - We are currently recruiting people from Victoria for this study, and are applying for funding to recruit people from Tasmania as well

# Acknowledgments

4,500 people with MS from Australia and NZ who agreed to participate in this study

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## Members of the ANZgene Consortium

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Preethi Guru  
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Simon Foote

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Laura Johnson  
Judith Field  
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