Genome-wide association studies II: Identifying genetic associations with complex traits

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MSc Global Health Science and Epidemiology

Genetic Epidemiology Module

Wednesday 5th Mar 2025

Learning objectives

Understand a genome-wide association study (GWAS) and the concept of a hypothesis-free approach to studying genetic associations.

Have a working knowledge of the different steps involved in the conduct of GWAS, including study design, quality control and basic analyses.

Be able to interpret and critically appraise evidence from genome-wide association studies.

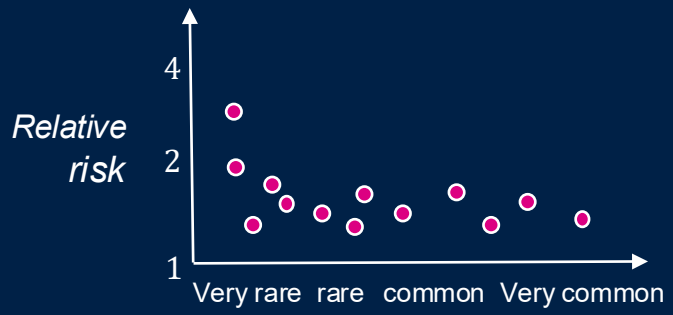
Understand the relevance of replication, meta-analysis and consortia, and multi-ancestry approaches, in genome-wide association studies.

Appreciate the use of post-GWAS analyses including fine mapping, gene and pathway analyses, and the concept of causal variants.

Main points in this lecture

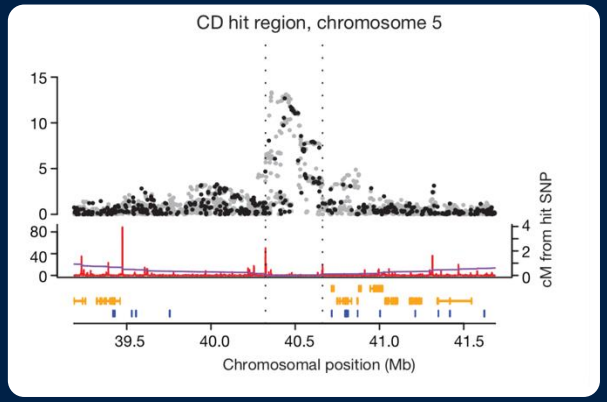
How polygenic do traits get, anyway?

Polygenicity – Genetic architecture - Consortia & Meta-analysis – GWAS trajectory

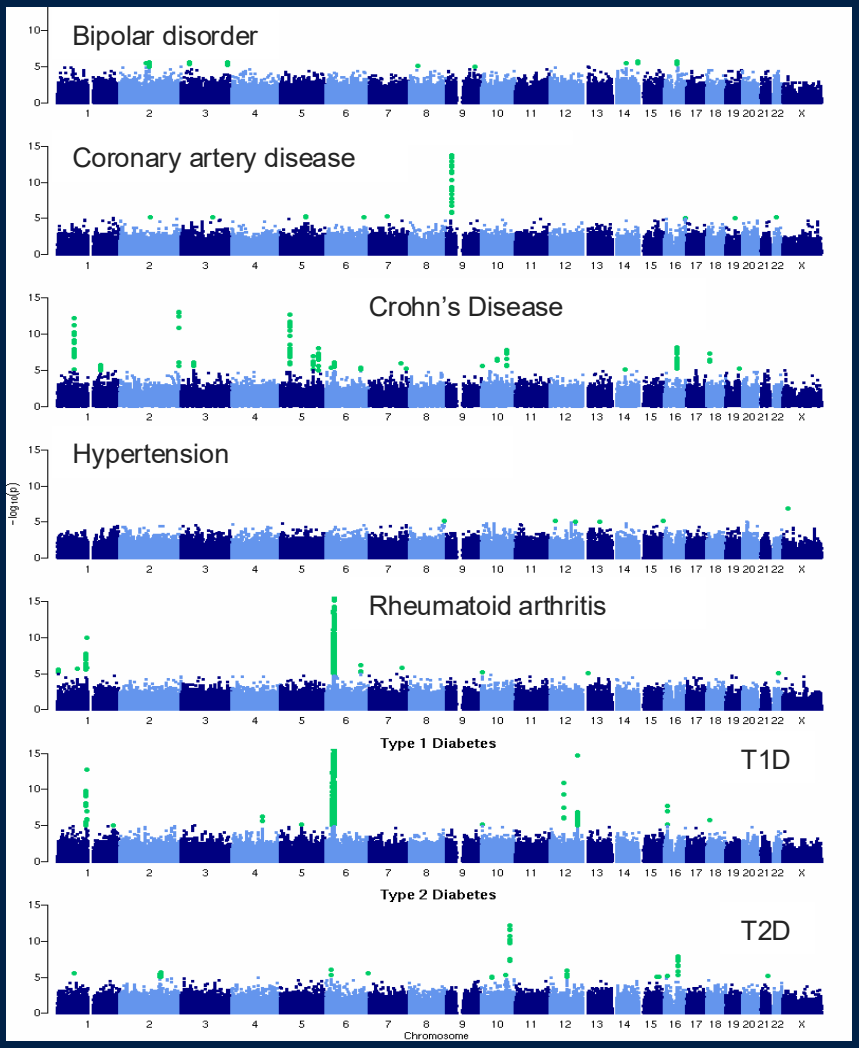


Extracting biological information from GWAS

Pathway analysis – fine mapping - pleiotropy

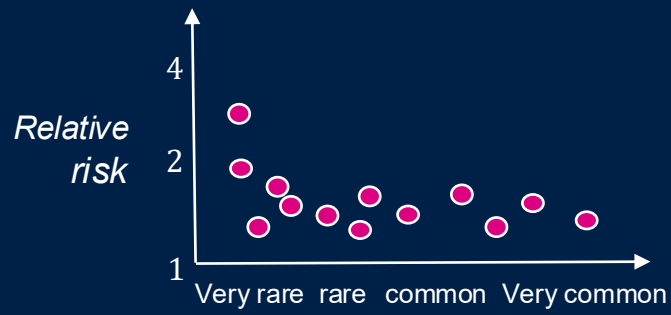


Actual results
from the Wellcome Trust Case-Control Consortium study:



Additive model, N=2,000 cases + 3000 controls per phenotype

My cartoon:



"Common variant, common trait" hypothesis

How polygenic do they get?

Maybe we haven't found them all - how could we find more?

Remember the formula

$$\text{Standard error}(\log OR) \approx \frac{1}{\sqrt{2N \times f(1-f) \times \phi(1-\phi)}}$$

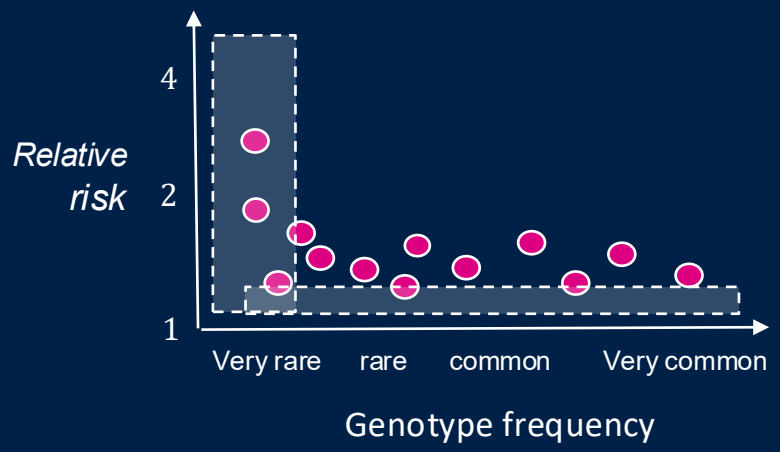
N = sample size*

f = frequency of allele

ϕ = proportion of cases

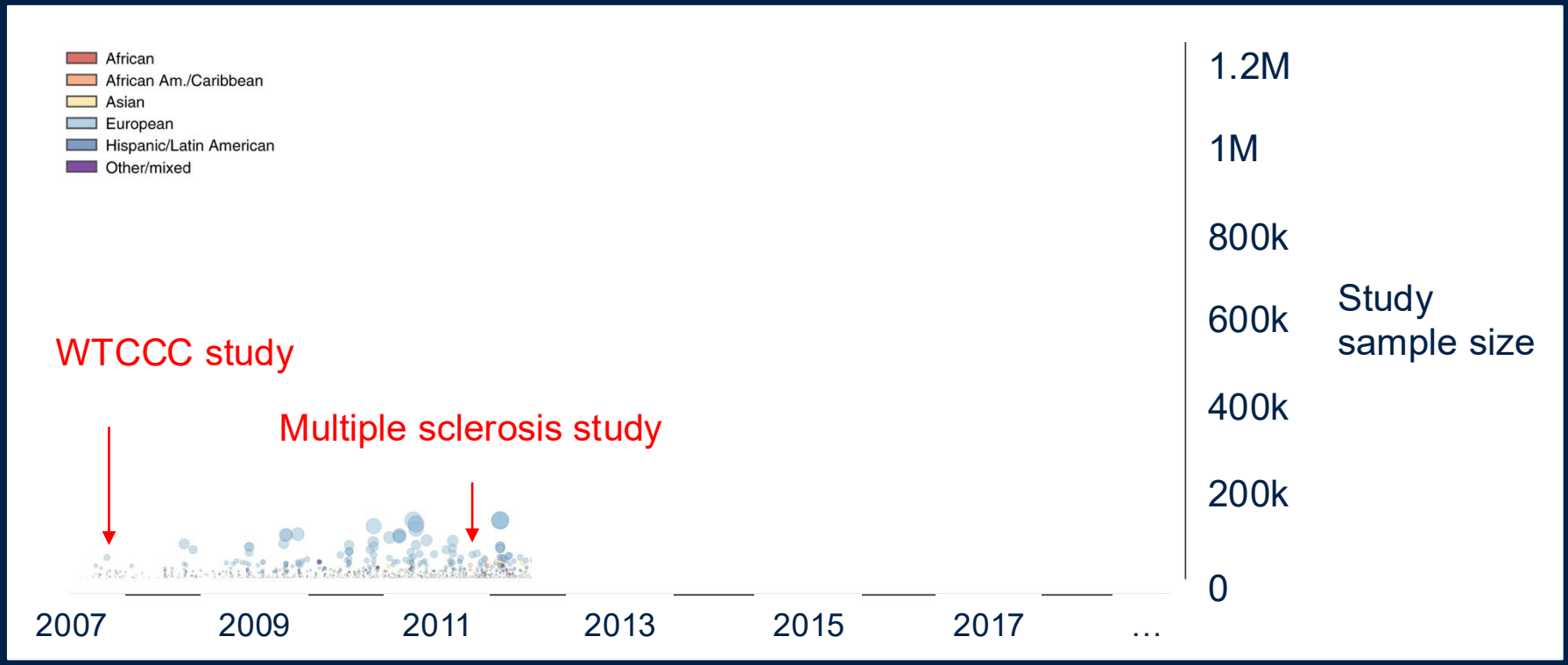
To find more associations we should:

increase the sample size



*Here expressed for additive model of association (each allele copy adds to the signal)

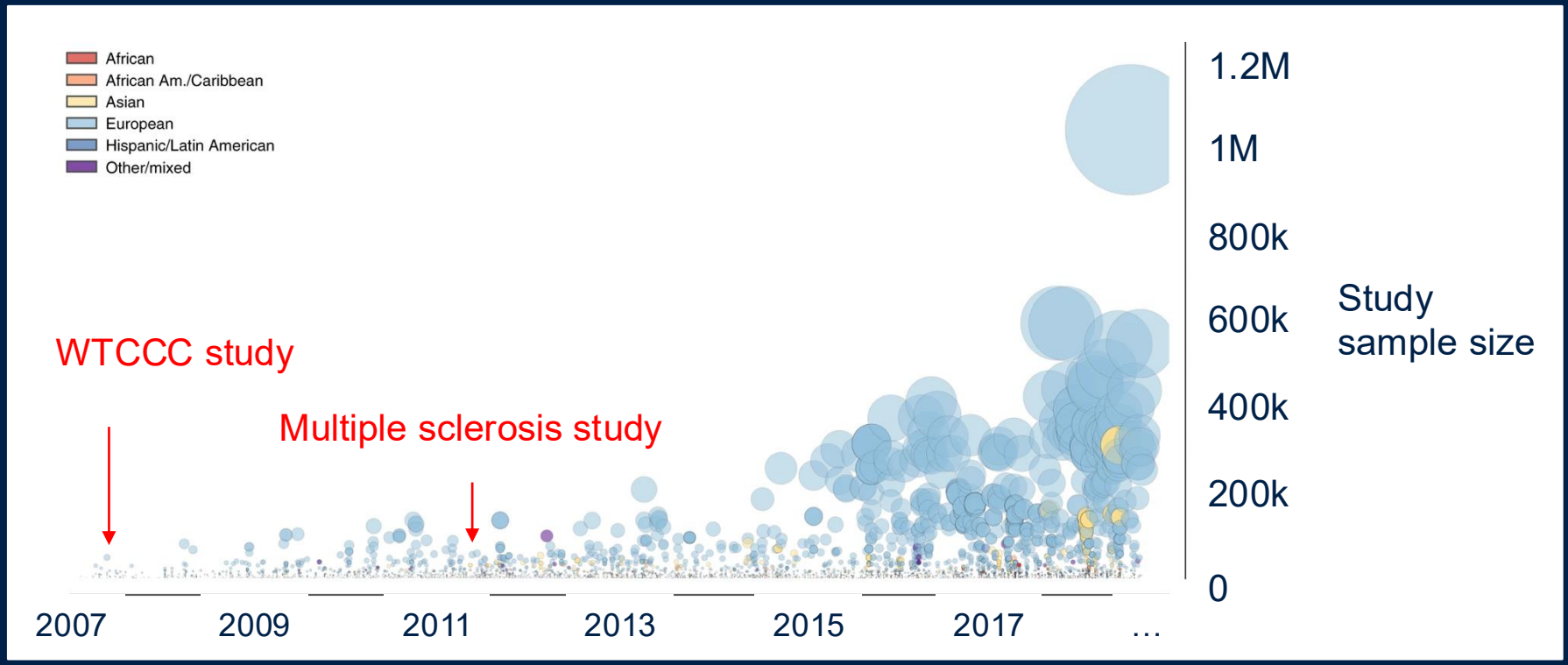
GWAS revolution



Mills & Rahal, "*A scientometric review of genome-wide association studies*", Communications Biology 2019

NHGRI GWAS Catalog: https://www.ebi.ac.uk/gwas/

GWAS revolution



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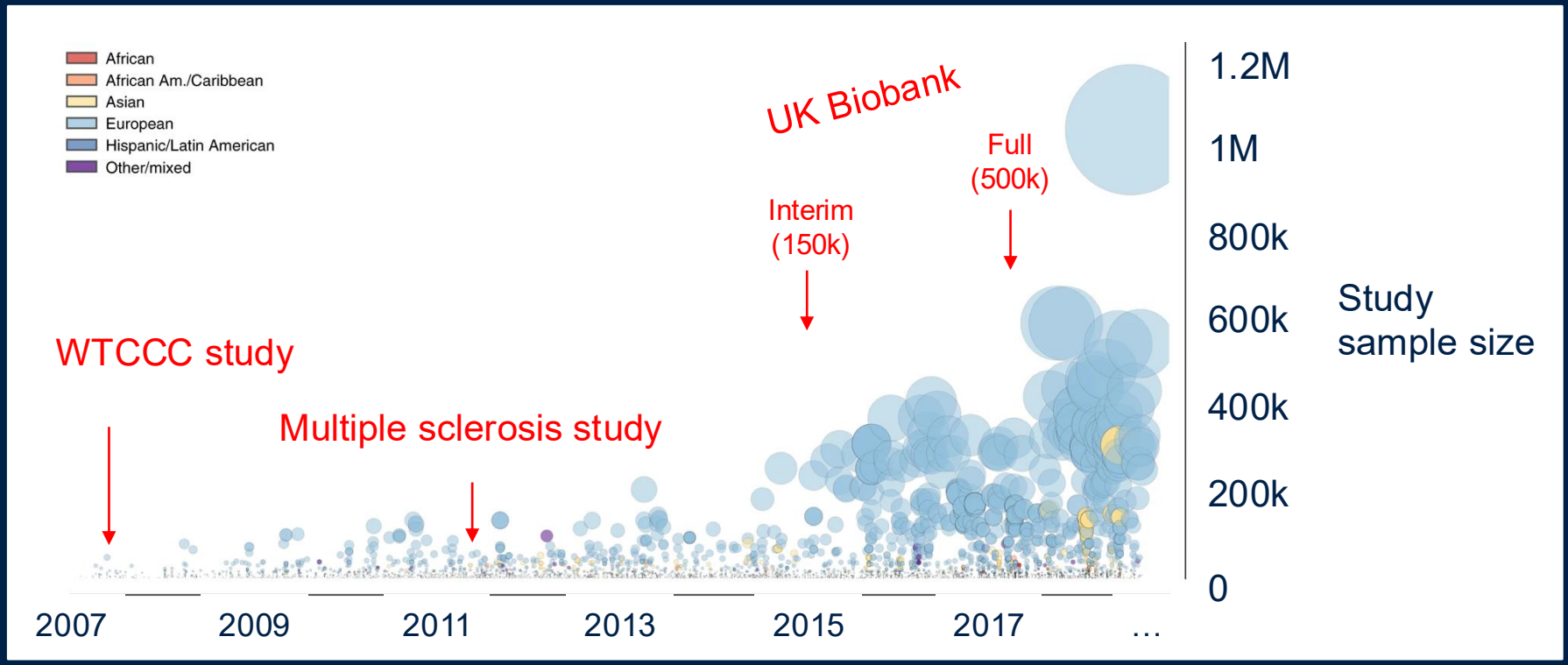

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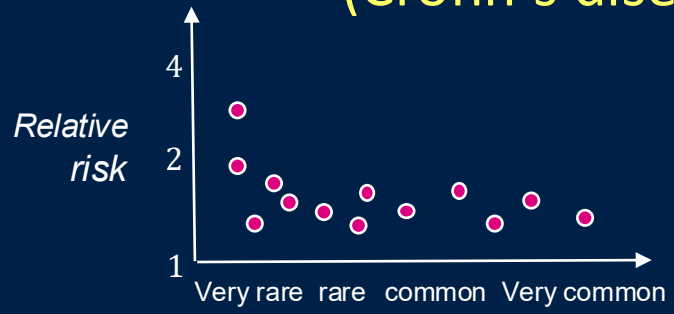
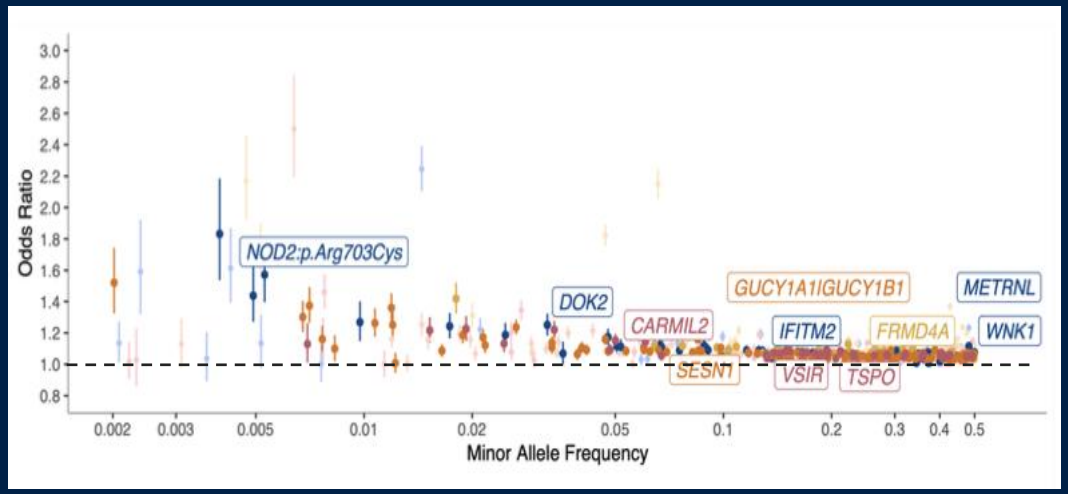
Inflammatory bowel disease

(Crohn's disease and ulcerative colitis)





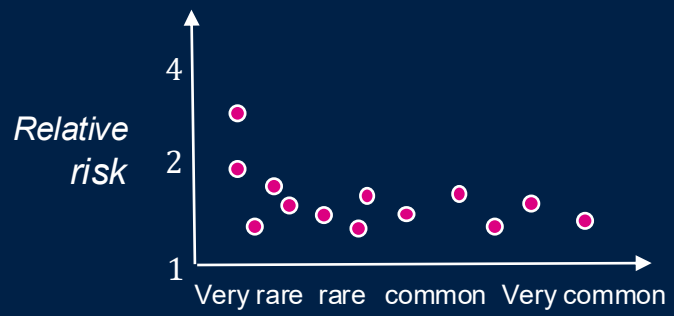
N = 125,992 IBD cases
1.2 million controls

> 600 association signals.

Abstract citation ID: jjad212.0008
OP08
Multi-ancestry genome-wide association study of inflammatory bowel disease identifies 125 novel loci and directly implicates new genes in disease susceptibility

L. Fachal[1], on behalf of the International IBD Genetics Consortium
[1]*Wellcome Sanger Institute, Human Genetics, Hinxton- Saffron Walden, United Kingdom*

Type 2 diabetes



N = 74,000 T2D cases
And 824,000 controls

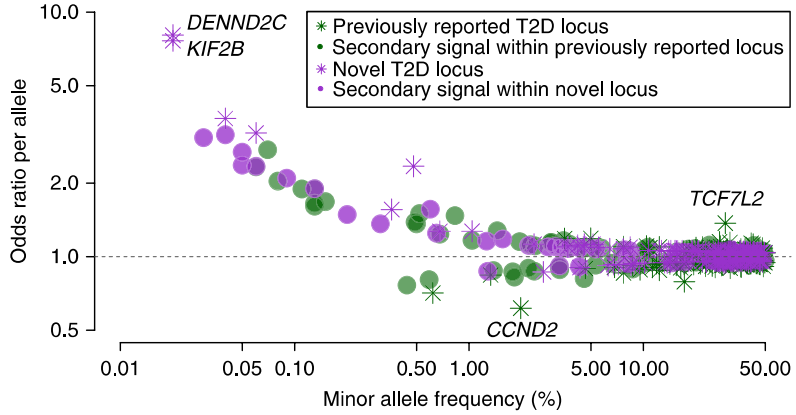


Fig. 5 | The relationship between effect size and MAF. Conditional- and joint-analysis effect size (*y* axis) and MAF (*x* axis) for 403 conditionally independent SNPs. Previously reported T2D-associated variants are shown in green, and novel variants are shown in purple. Stars and circles represent the 'strongest regional lead at a locus' and 'lead variants for secondary signals', respectively.

403 signals

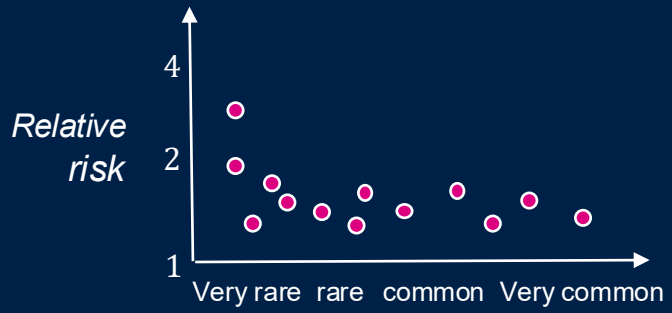
"conditionally independent" meaning some of them overlap the same regions

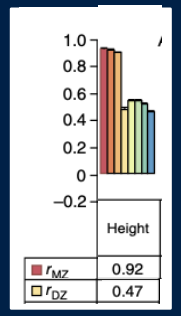
nature genetics
ARTICLES
https://doi.org/10.1038/s41588-018-0241-6

Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps

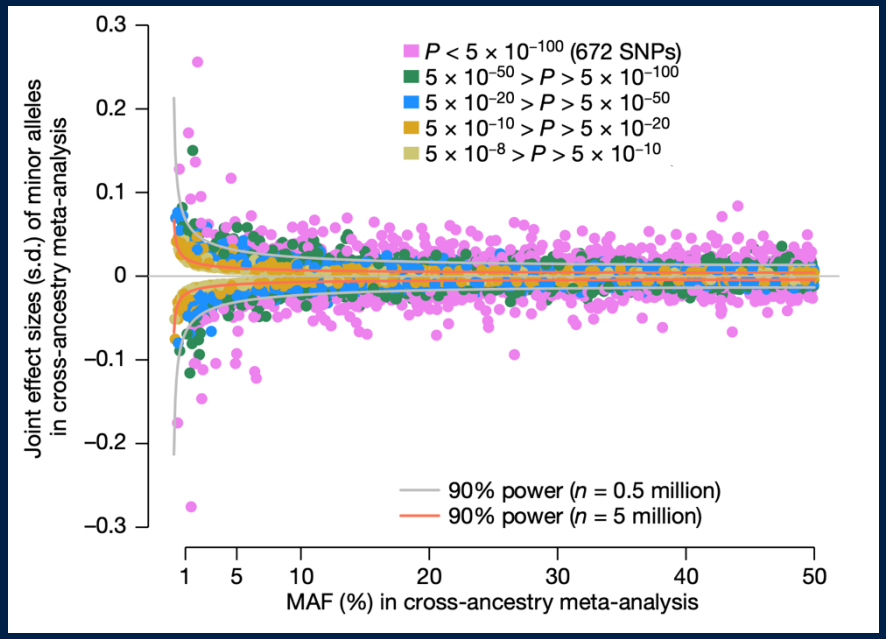
GWAS of human height

In 5.4 million individuals

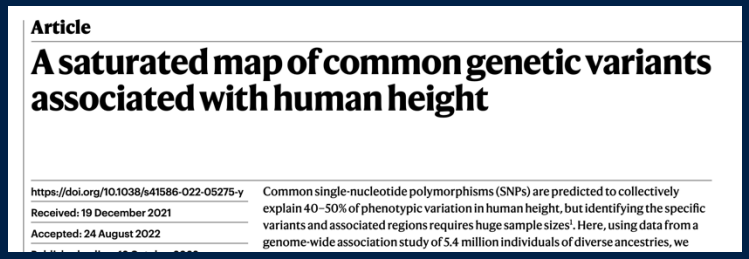




~90% heritability



? signals



Article

A saturated map of common genetic variants associated with human height

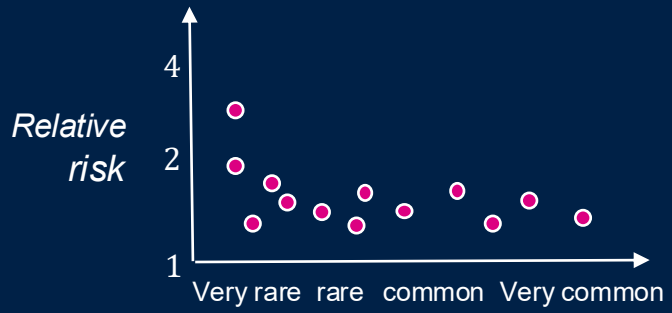
https://doi.org/10.1038/s41586-022-05275-y
Received: 19 December 2021
Accepted: 24 August 2022

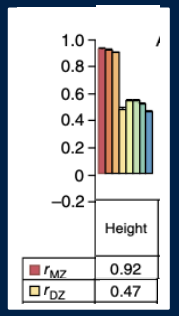
Common single-nucleotide polymorphisms (SNPs) are predicted to collectively explain 40–50% of phenotypic variation in human height, but identifying the specific variants and associated regions requires huge sample sizes[1]. Here, using data from a genome-wide association study of 5.4 million individuals of diverse ancestries, we

N = 5.4 million

GWAS of human height

In 5.4 million individuals





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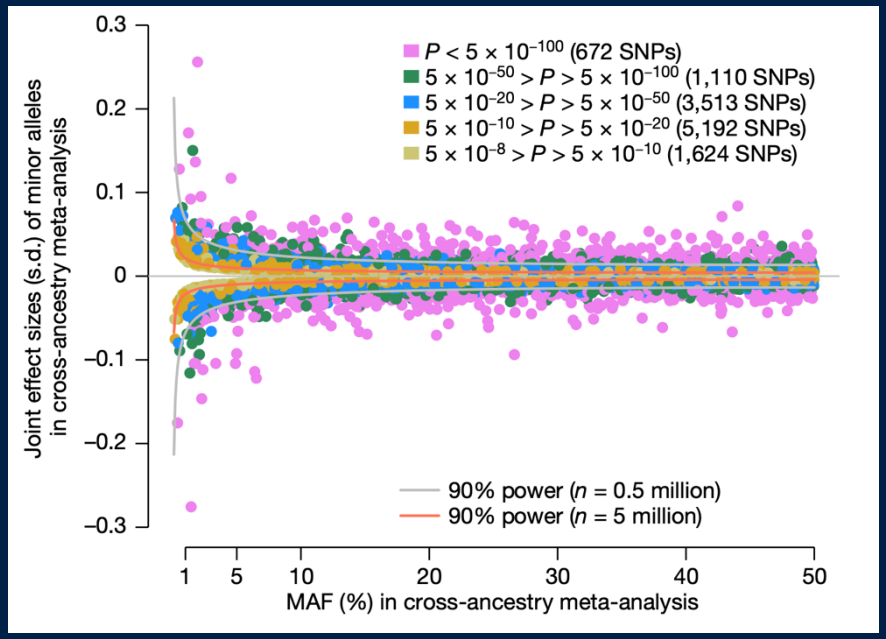
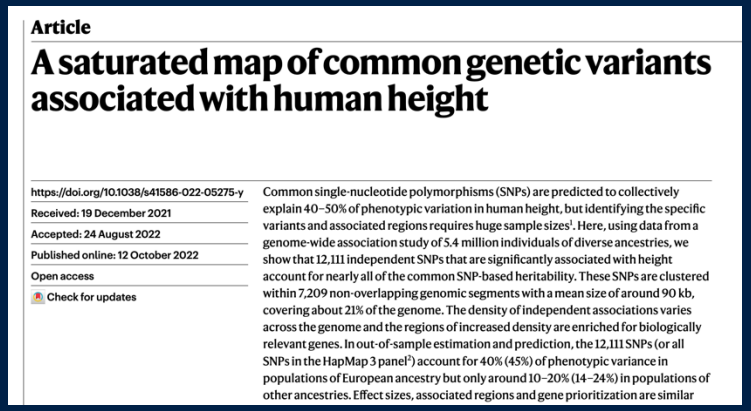


N = 5.4 million

12,111 independent signals

Collectively explaining 50% of heritability



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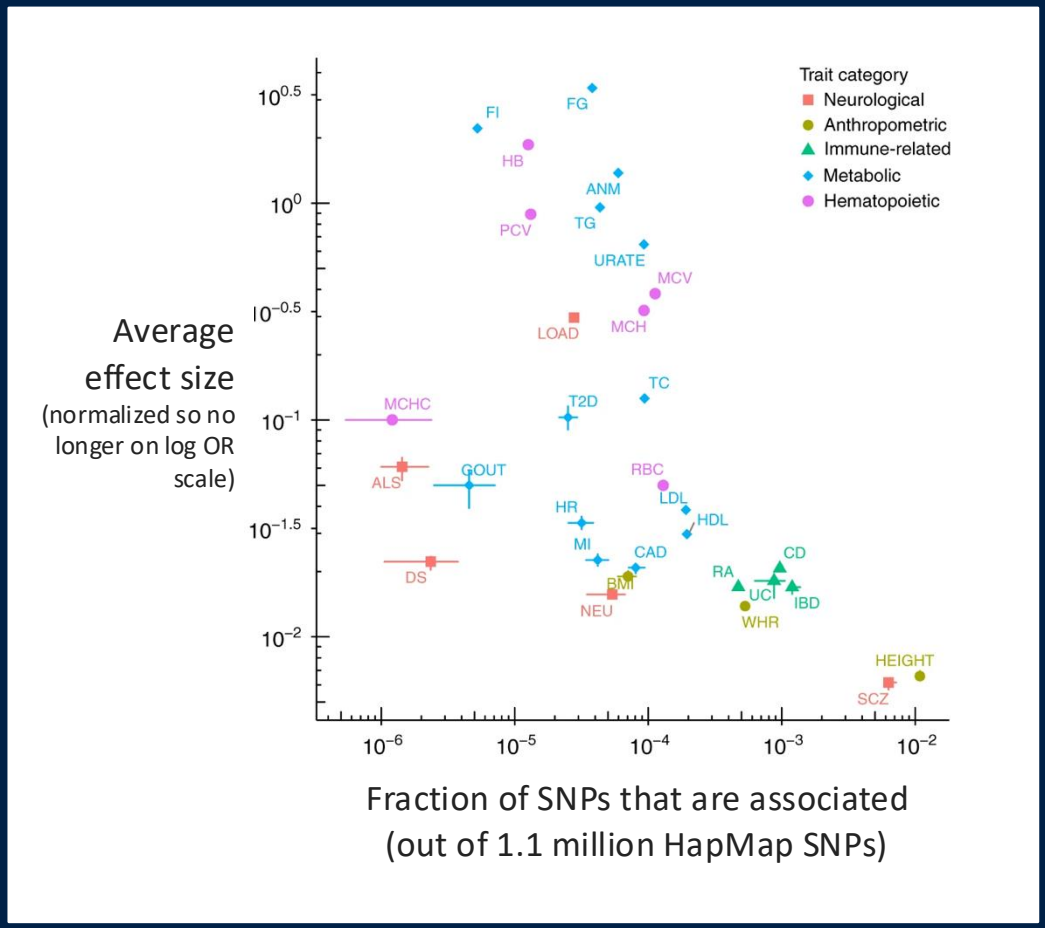
Check for updates

Common single-nucleotide polymorphisms (SNPs) are predicted to collectively explain 40–50% of phenotypic variation in human height, but identifying the specific variants and associated regions requires huge sample sizes[1]. Here, using data from a genome-wide association study of 5.4 million individuals of diverse ancestries, we show that 12,111 independent SNPs that are significantly associated with height account for nearly all of the common SNP-based heritability. These SNPs are clustered within 7,209 non-overlapping genomic segments with a mean size of around 90 kb, covering about 21% of the genome. The density of independent associations varies across the genome and the regions of increased density are enriched for biologically relevant genes. In out-of-sample estimation and prediction, the 12,111 SNPs (or all SNPs in the HapMap 3 panel[2]) account for 40% (45%) of phenotypic variance in populations of European ancestry but only around 10–20% (14–24%) in populations of other ancestries. Effect sizes, associated regions and gene prioritization are similar

They collectively explain ~ 50% of heritability

In European ancestry people

Comparing across traits



With all this data it's possible to fit more sophisticated models that estimate the amount of polygenicity across traits.

ARTICLE
DOI: 10.1038/s41467-018-06805-x OPEN

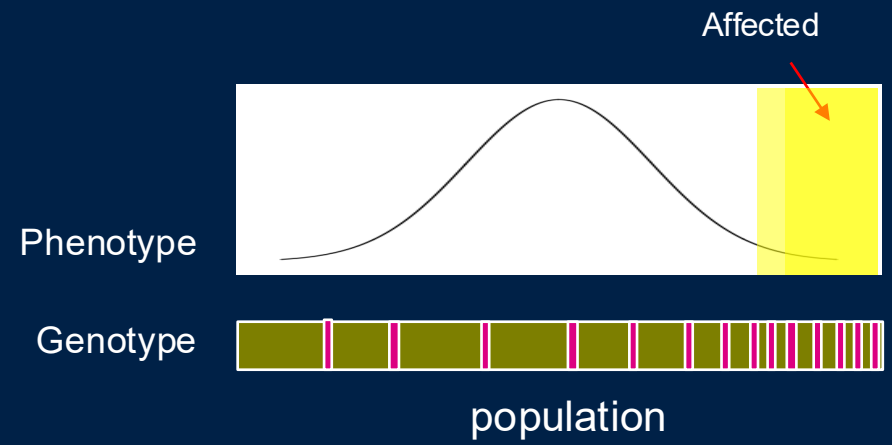
Large-scale genome-wide enrichment analyses identify new trait-associated genes and pathways across 31 human phenotypes

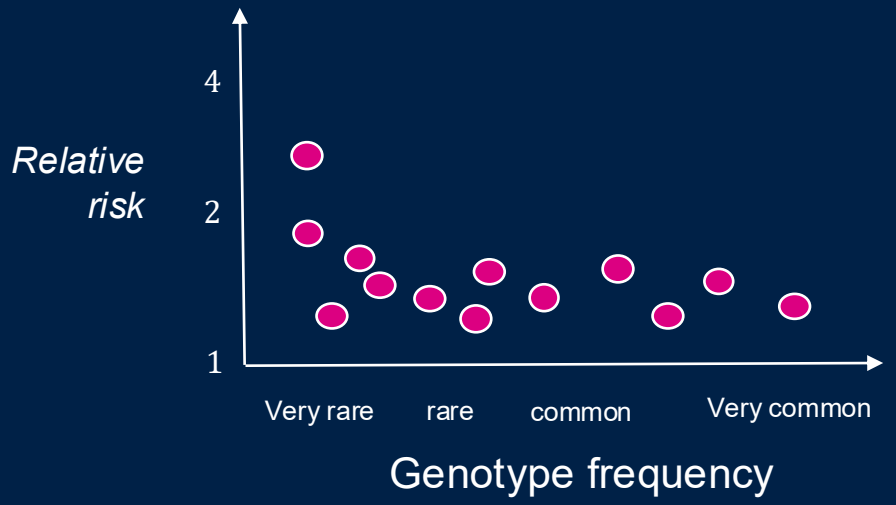
Xiang Zhu[1,2] & Matthew Stephens[2,3]

GWAS revolution

NHGRI GWAS Catalog: https://www.ebi.ac.uk/gwas/diagram

Common variant, common disease hypothesis





A **complex trait**.
Caused by many factors, each having a small overall effect. Including

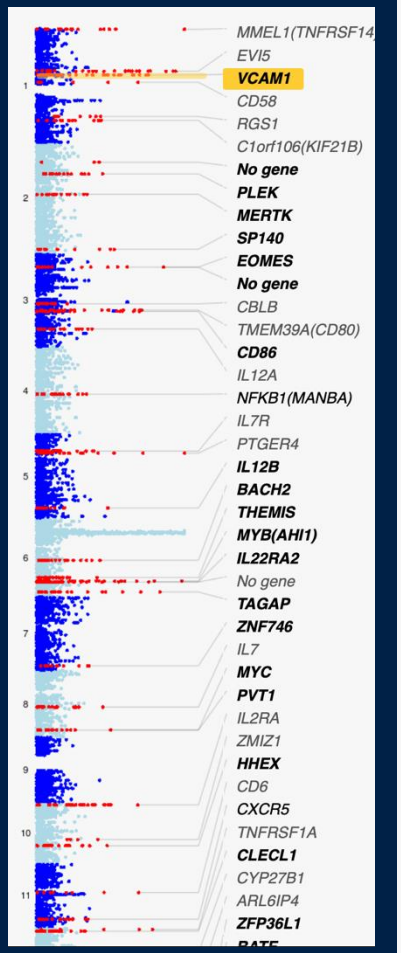
- Many genetic variants, including common ones
- Environmental factors
- Gene-environment or gene-gene interactions
- ...

How are these studies possible?

Consortia and meta-analysis

Consolidation question from last lecture

WTCCC2 GWAS of multiple sclerosis (9,772 cases and 7,376 controls).



For further information about terms used below, hover over the red question marks.

Region

dbSNP id:	rs11581062
status:	novel association
physical position:	01:101,180,107
association region:	01:100,983,315-101,455,310
functional tag:	*N/A*
nearest gene:	*SLC30A7*
candidate gene:	*VCAM1**

Signal

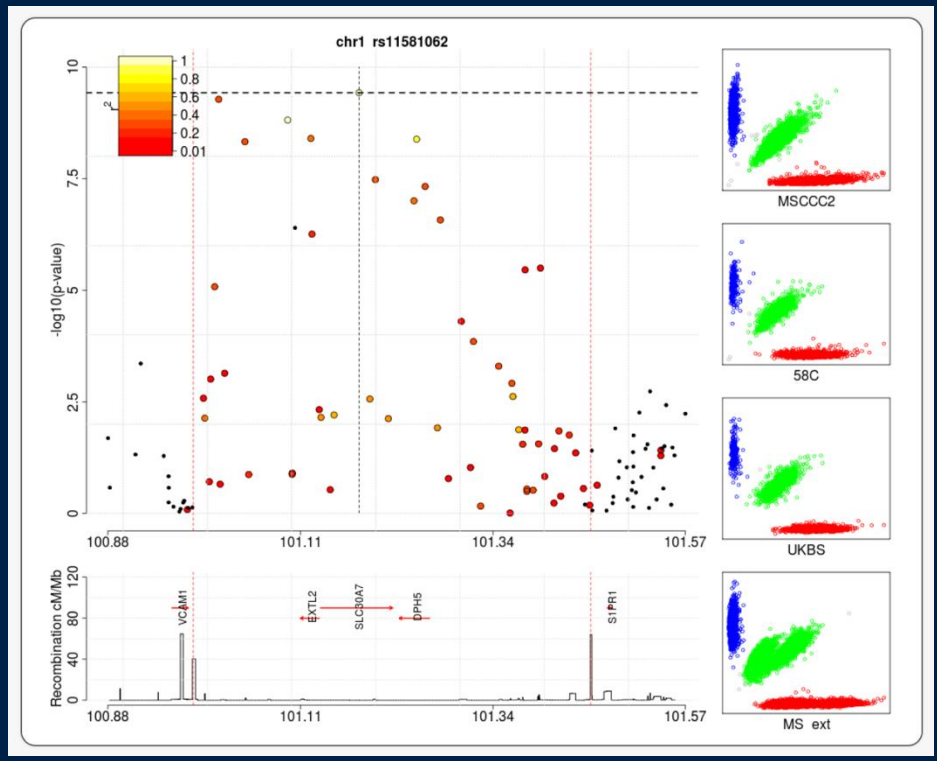
p-value discovery:	3.7e-10
OR discovery (95% CI):	1.13 (1.09-1.18)
p-value replication:	4.20e-02 (one-sided)
OR replication (95% CI):	1.07 (0.99-1.15)
p-value combined:	2.50e-10
OR combined (95% CI):	1.12 (1.1-1.13)
Risk (non-risk) allele:	G(A)

Allele frequencies

Country	controls / cases	control / case frequency
Australia	- / 647	- / 0.32
Belgium	- / 544	- / 0.33
Denmark	- / 332	- / 0.32
Finland	2165 / 581	0.23 / 0.24
France	347 / 479	0.31 / 0.34
Germany	1699 / 1100	0.29 / 0.31
Ireland	- / 61	- / 0.34
Italy	571 / 745	0.30 / 0.33
Norway	121 / 953	0.26 / 0.28
Poland	- / 58	- / 0.27
Spain	- / 205	- / 0.36
Sweden	1928 / 685	0.27 / 0.28
UK	5175 / 1854	0.29 / 0.32
USA	5370 / 1382	0.29 / 0.32

Proximal genes

DPH5, *EXTL2*, *S1PR1*, *SLC30A7*, *VCAM1**

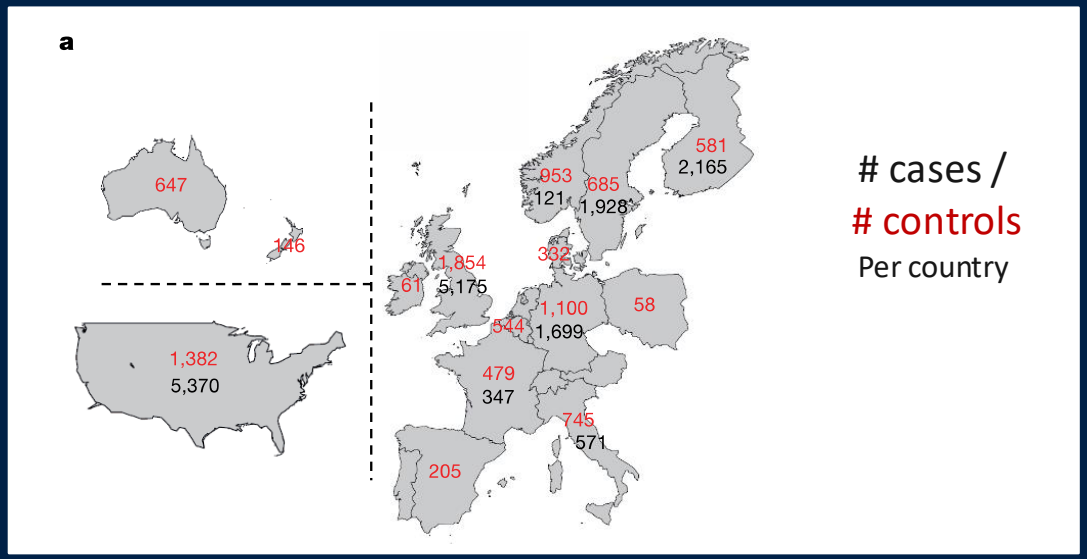


Can you explain?

Consortia and meta-analysis

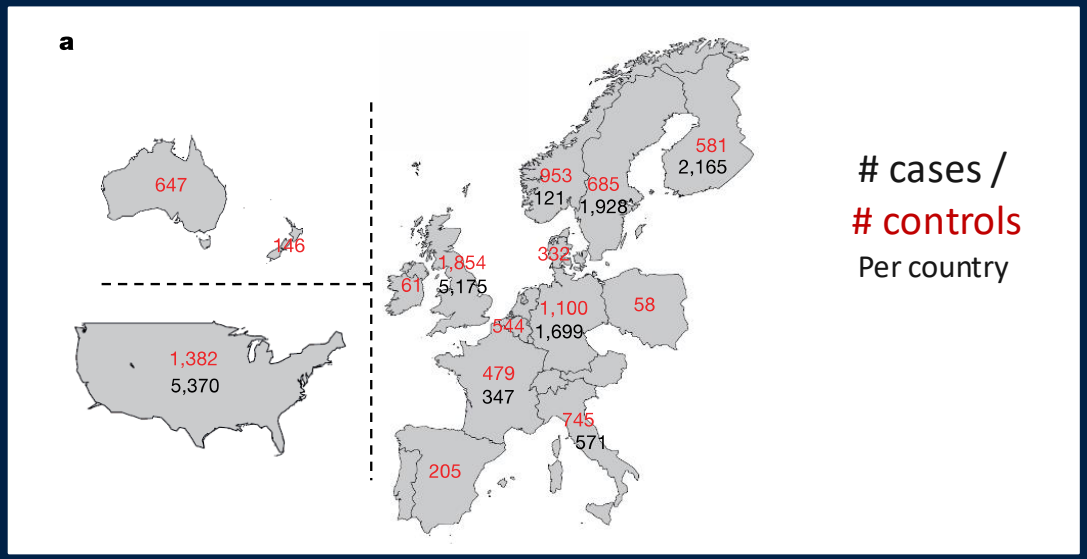
To generate such large sample sizes for “common” (but still relatively rare) diseases, requires setting up large multi-centre collaborations. This is fun to be involved in but comes with its own analysis challenges….

Dealing with population structure



This study suffered from a key problem. Can you see what it is?

Dealing with population structure



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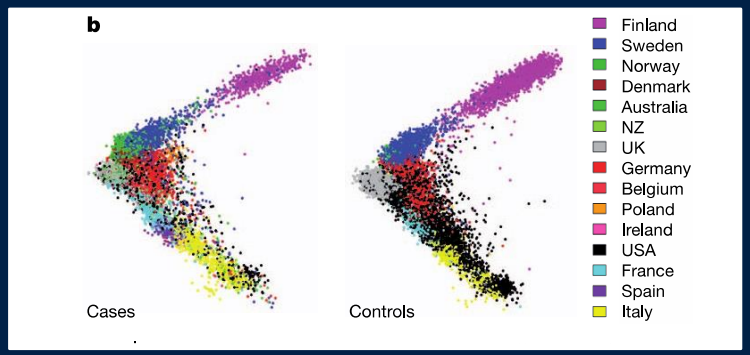
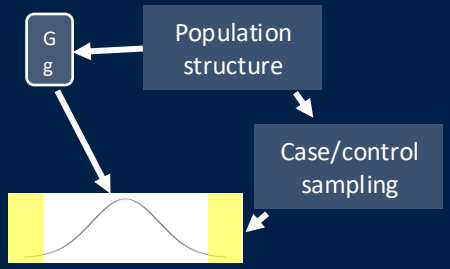


First two "principal components" obtained purely from the genotypes

Case-control sampling is correlated with genome-wide genetic variation.

"Confounding by population structure"

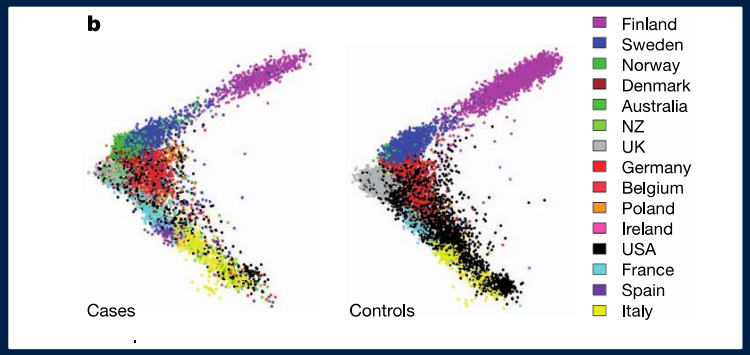


Population structure: solutions

Instead of simple 2x2 table

1. **Regression including principal components**

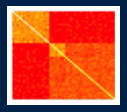
$$\text{outcome} \sim \text{genotype} + PCs$$



Plot of first two principal components obtained from the genome-wide genotypes

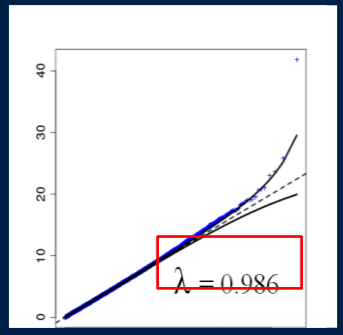
Uses just the strongest directions of variation in relatedness (population structure)

2. Linear mixed model

$$\text{outcome} \sim \text{genotype} +$$ 

Include a **genetic relatedness matrix computed from genome-wide genotypes** in the association test

Uses the **entire matrix** of relationships



MS study

Most p-values are now not inflated

Anatomy of an association analysis

All GWAS should report data in a way that can be re-used by future studies.
This study used several previous GWAS to conduct replication. All the details are given in a supplementary table:

		WAS + replicatio		GWAS			UK only GWAS		non-UK only GWAS		combined replication		eneMSA NL replicatio			ene MSA US replicatio			eneMSA CH replicatio			ANZ replication			BWH replication		
Gene	RiskAllele	pval	OR (95% CI	pval	OR (95% CI	log10 (BayesFacto	pval	OR (95% CI	pval	OR (95% CI	pval**	OR (95% C	pval**	OR (95% CI	inf	pval**	OR (95% CI	inf	pval**	OR (95% CI	inf	pval**	OR (95% CI	inf	pval	OR (95% CI	inf
MMEL1	C	1.00E-14	1.14 (1	3.10E-14	1.16 (1	11.39	0.0073	1.12 (1	7.10E-13	1.17 (1	0.0085	1.08 (1.01-1.15)	0.26	1.1 (0.	0.94	0.18	1.1 (0.	1.01	0.24	1.11 (0	1.03	0.006	1.15 (1	1	0.41	1.02 (0	
EVI5	A	5.80E-15	1.15 (1	6.50E-12	1.15 (1	9.15	2.90E-05	1.2 (1.	2.70E-08	1.14 (1	1.00E-04	1.14 (1.06-1.22)	0.088	1.23 (0	1.05	0.59	0.97 (0	0.91	0.71	0.92 (0	0.94	0.023	1.12 (1	0.97	0.0059	1.18 (1	1
SLC30A7	G	2.50E-10	1.12 (1	3.70E-10	1.13 (1	7.43	0.00047	1.16 (1	1.70E-07	1.13 (1	0.042	1.07 (0.99-1.15)	0.57	0.99 (0	1.01	0.095	1.09 (0	0.99	0.013	1.18 (0	0.91	0.57	0.99 (0	1.01	0.095	1.09 (0	0
EXTL2	A	4.00E-08	1.09 (1	3.70E-07	1.1 (1.	4.52	0.00096	1.14 (1	6.00E-05	1.08 (1	0.017	1.08 (1.01-1.15)	0.025	1.11 (1	1	0.088	1.09 (0	1.01	0.45	1.01 (0	0.88	0.025	1.11 (1	1	0.088	1.09 (0	1.

Discovery and overall data as on web page

Evidence for the same effect direction was seen separately in both arms of the discovery…

…and in the combined replication…

…and in most of the individual replication studies.

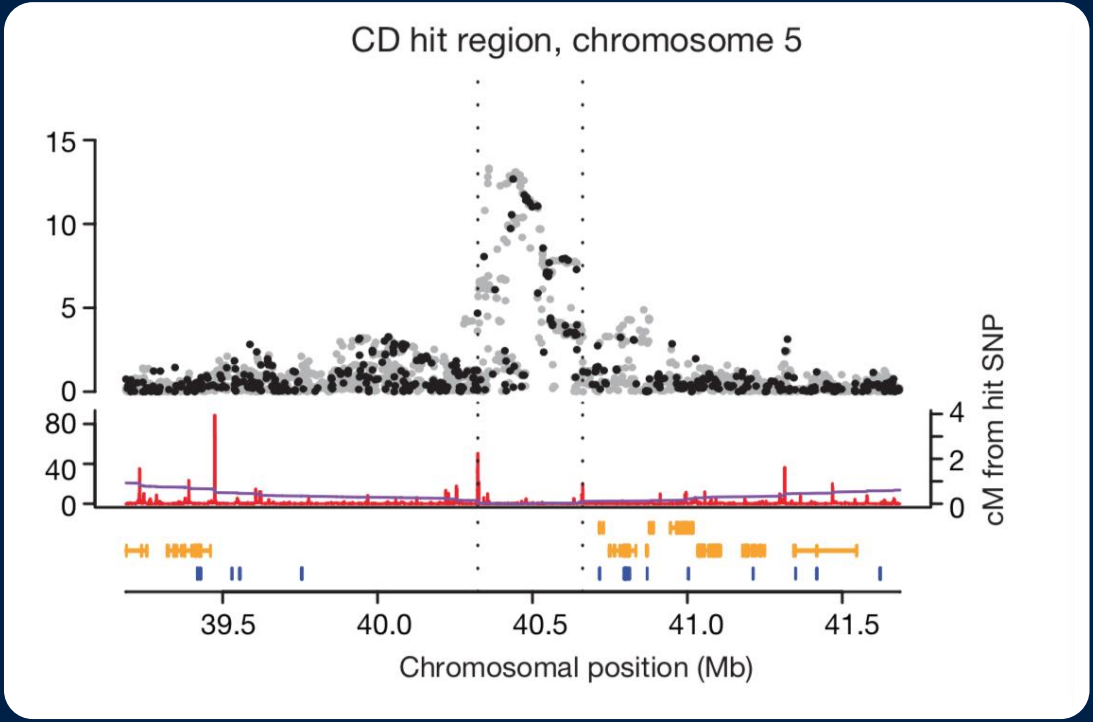
This is a common analysis approach: to gain sample size, use meta-analysis to combine results across several component studies. Then look for consistency between the studies.

$$v_{meta} = 1/\left(\sum_i \frac{1}{v_i}\right) \qquad \beta_{\text{meta}} = \left(\sum_i \frac{\beta_i}{v_i}\right) \times v_{meta}$$

(Where v denotes squared standard error)

" Inverse variance weighted fixed-effect meta-analysis", gives results approximately equal to joint analysis of genotype data.

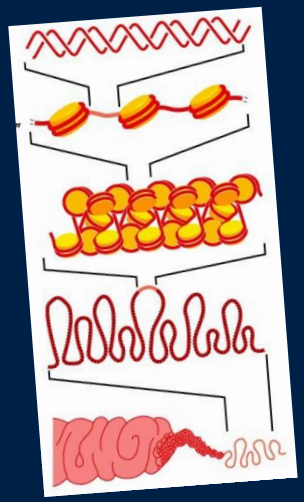
We now have thousands of GWAS signals across thousands of traits. What do they teach us about the underlying biology?



?

The circle of genetic causation

DNA gets physically packaged up into chromosomes...



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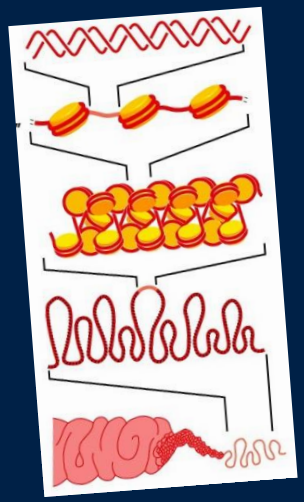
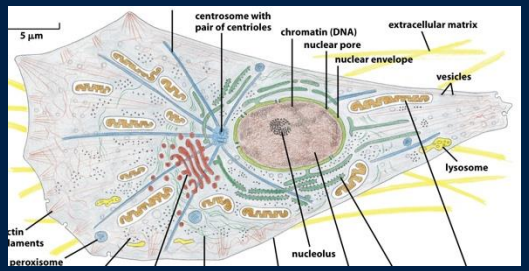
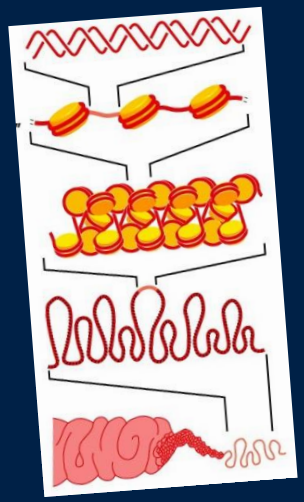


...inside cells, where it is **transcribed** to form proteins and other molecules...



The circle of genetic causation

DNA gets physically packaged up into chromosomes...



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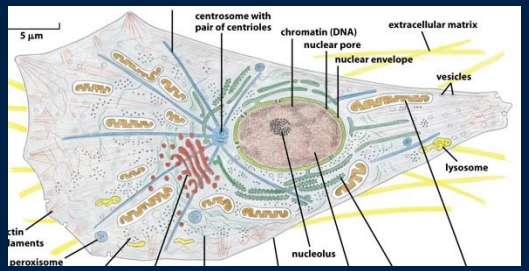
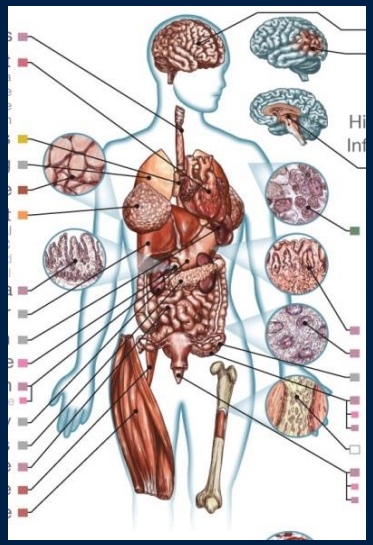


...that affect how the cells behave, forming different organs...



...that combine to make individuals...

The circle of genetic causation

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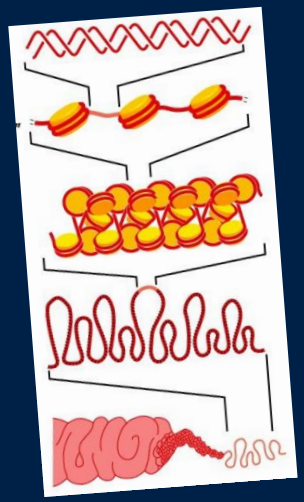

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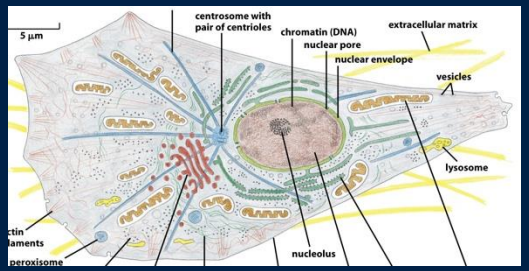

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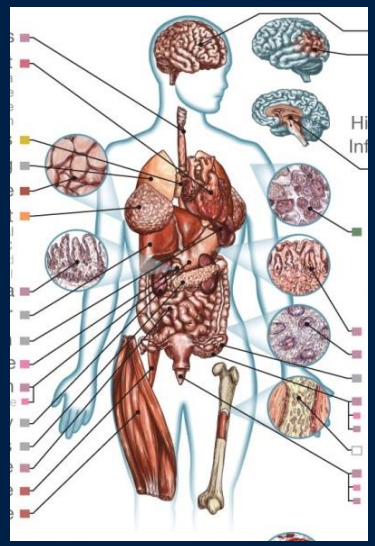

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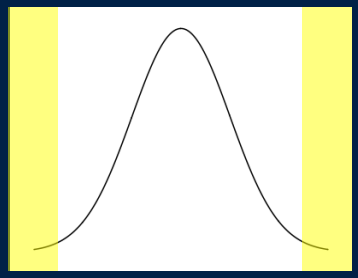
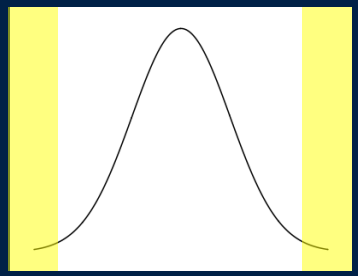


...whose success is affected by the traits they have...

The circle of genetic causation

...passing on DNA, with **mutations** and **recombination**, to new generations...



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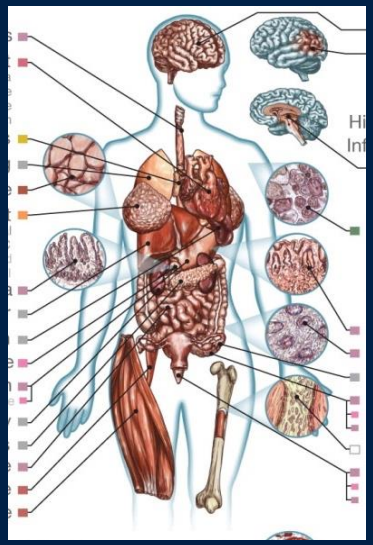

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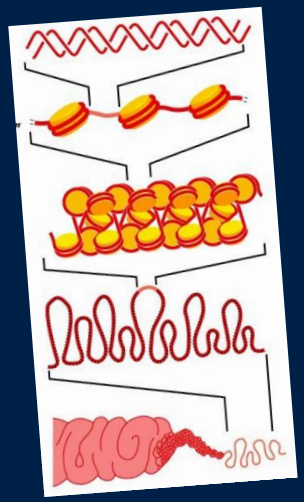
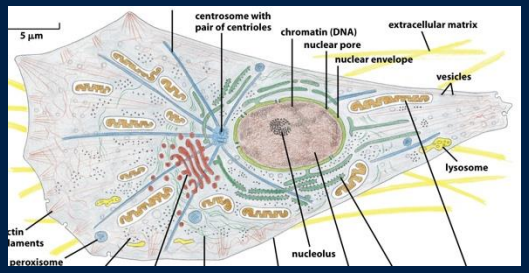


There is complex biology at all stages

...inside cells, where it is **transcribed** to form proteins and other molecules...



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The circle of genetic causation

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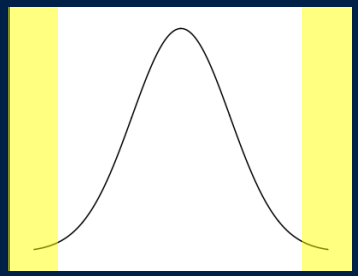
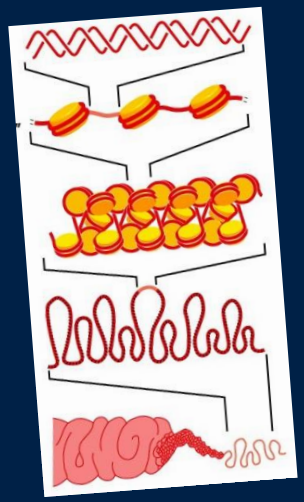


...whose success is affected by the traits they have...

microarrays, genome sequencing

Clinical phenotype measurements

...that gets physically packaged up into chromosomes...

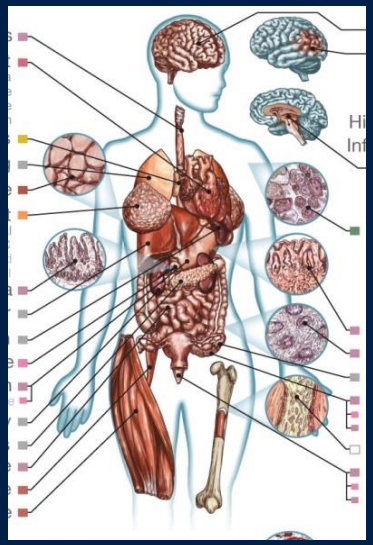


Chromatin state marker assays, ChIP-seq, ...

There is complex biology at all stages

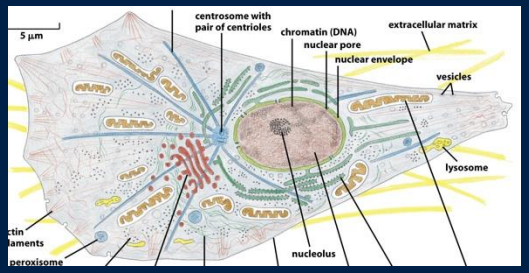
and we can measure it

Biomarker measurements



RNA-seq, spectroscopy, antibody binding

...that combine to make individuals...



...inside cells, where it is **transcribed** to form proteins and other molecules...

...that affect how the cells behave, forming different organs...

Gaining biological knowledge from GWAS

There are several ways we can try to translate knowledge of associations into new biological insights. I will try to describe a few of these.

- Fine-mapping

Can we identify the actual causal variants underlying these associations, and hence discover specific proteins and disease pathways?

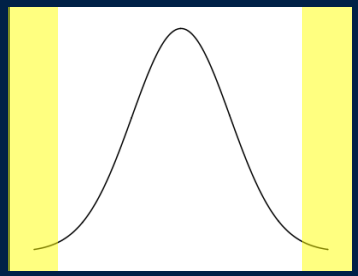
- Pathway analysis

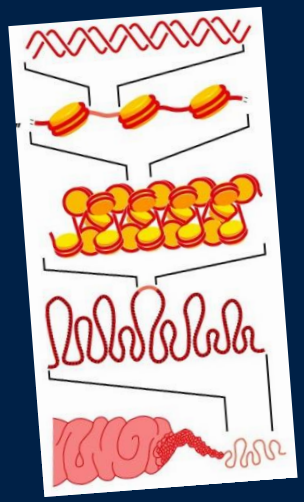
Even if we can't fine-map, we can still try to assess whether associations group into particular biological pathways that might shed light on biology

- Pleiotropy

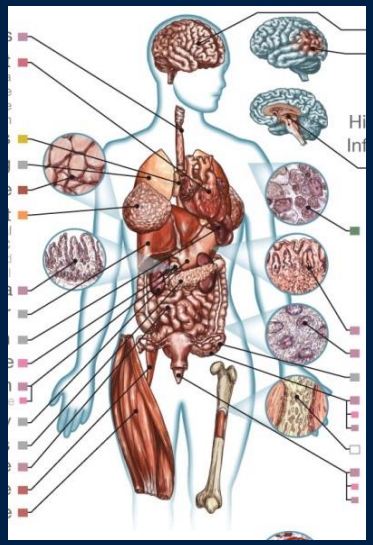
how are associations shared between traits?

The circle of genetic causation





Example 1: a pathway analysis



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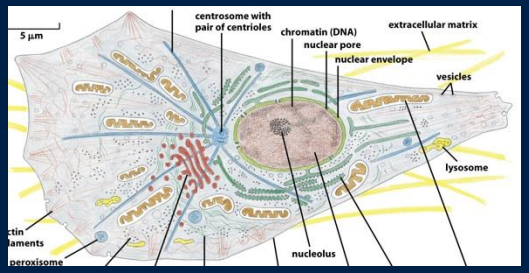


Pathway analysis

Pathway analyses and gene enrichment analysis seek to determine whether there is a statistical tendency for association signals to fall into known groups of related genes. These can be

- Known biological pathways (functional networks of proteins and molecules, performing known specific biological functions) – such as those available from the KEGG and Reactome databases

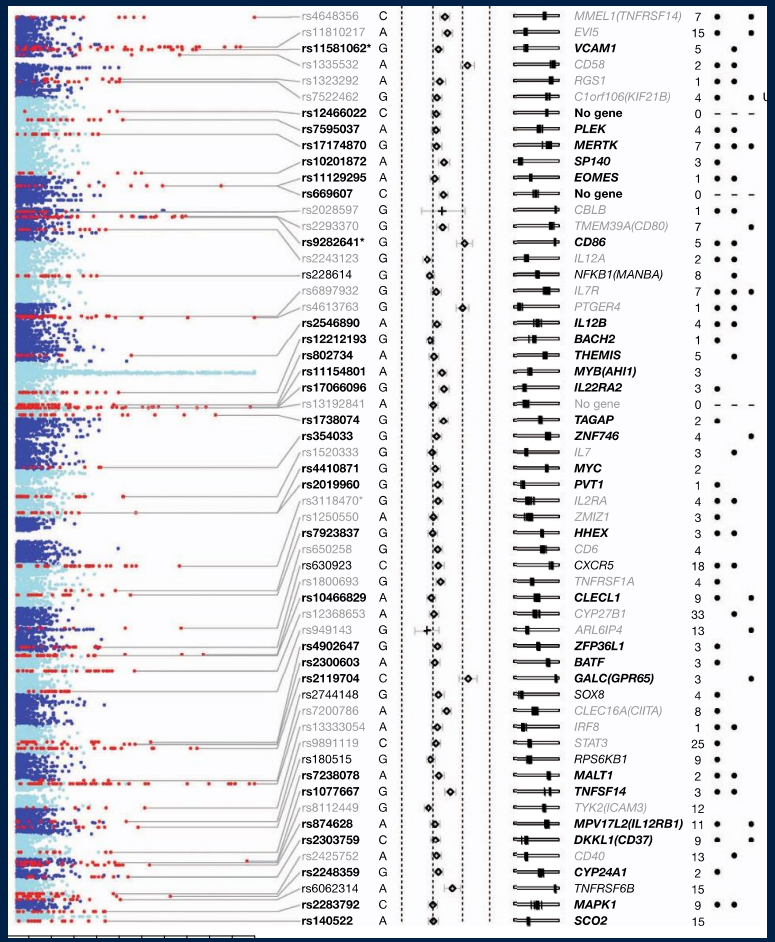
- More general classifications of genes by function, such as those from the Gene Ontology Project

A slightly different direction is to try to group signals by genome function – for example, do they lie in exons? Or gene promoters? Or in regulatory regions active in particular cells?

https://www.genome.jp/kegg/ https://reactome.org http://geneontology.org

Pathway analysis example

The primary cause of MS has typically been thought to be inflammation causing downstream neurodegeneration – with some debate about this. Can the GWAS of MS we discussed shed light on this?



Clinical and Experimental Neuroimmunology 1 (2010) 2–11

REVIEW ARTICLE

What drives disease in multiple sclerosis: Inflammation or neurodegeneration?

Hans Lassmann

Center for Brain Research, Medical University of Vienna, Vienna, Austria

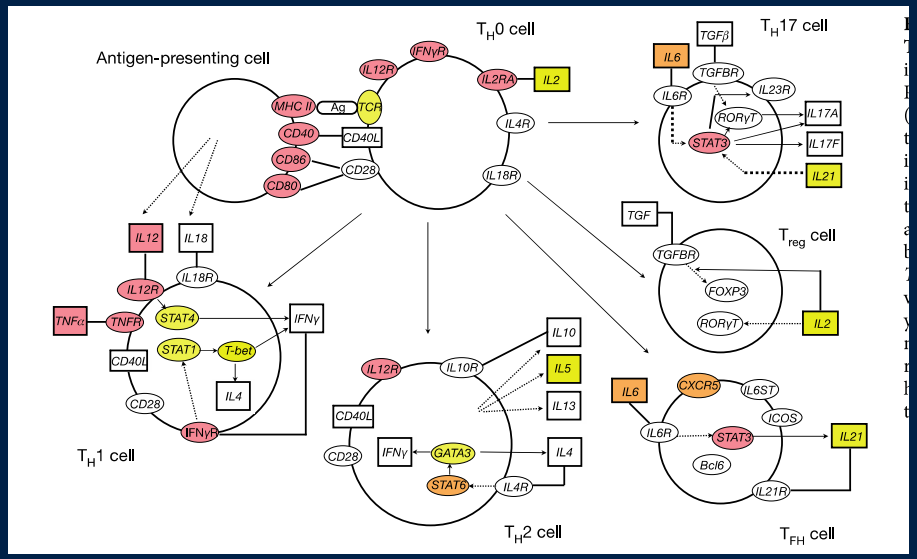
As the main figure shows, many of the association signals looked like they were near immune-system related genes.

www.well.ox.ac.uk/wtccc2/ms/

Pathway analysis example

We:

- Assigned SNPs to their nearest gene using the available annotation
- Used the Gene Ontology Project to classify genes into functionally related groups
- Conducted a statistical test (Fisher's exact test) to identify whether the nearest genes were enriched in each group.



T-helper-cell differentiation pathway
(from Ingenuity Pathway Analysis software)

Particularly strong enrichment was observed for immune system pathways – notably in "T cell activation and proliferation" ($P=1.9x10^{-9}$)

"Although GO immune system genes only account for 7% of human genes, in 30% of our association regions the nearest gene to the lead SNP is an immune system gene"

Published: 10 August 2011

Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis

The International Multiple Sclerosis Genetics Consortium & The Wellcome Trust Case Control Consortium 2

www.well.ox.ac.uk/wtccc2/ms/

Fine-mapping

"Fine-mapping" is the general term used for attempts to narrow down association signals to the underlying causal variants. A typical process involves:

- Gathering complete information on genetic variation in the region of interest – for example by deep-sequencing a large number of individuals. (Large databases such as gnomAD / TopMed now make this easier.)

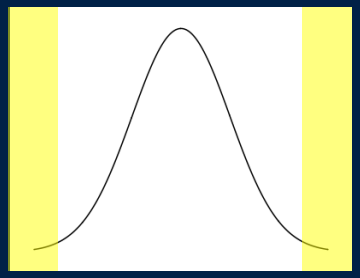
- Gathering information on genome function – including gene structure and regulatory regions.

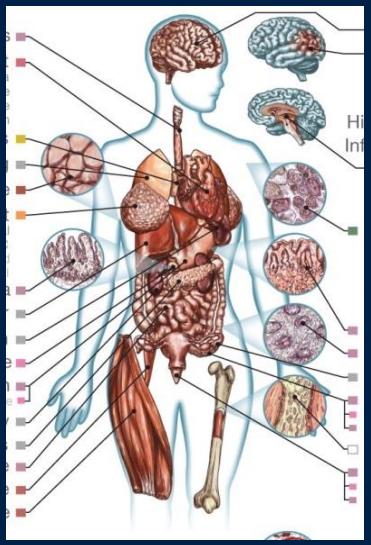
- Potentially leveraging data from different ancestral backgrounds, hoping that differences in LD patterns will help narrow down signals.

- Fitting models that attempt to parse apart multiple associations in the same region

Possible underlying mechanisms are pretty diverse and a healthy dose of genomic detective work is often needed.

The circle of genetic causation





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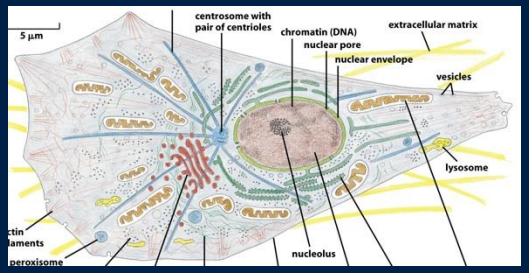
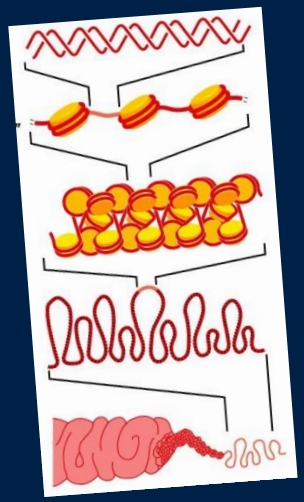
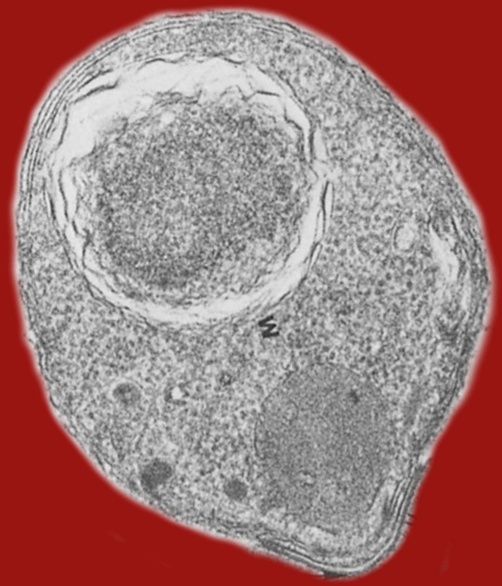


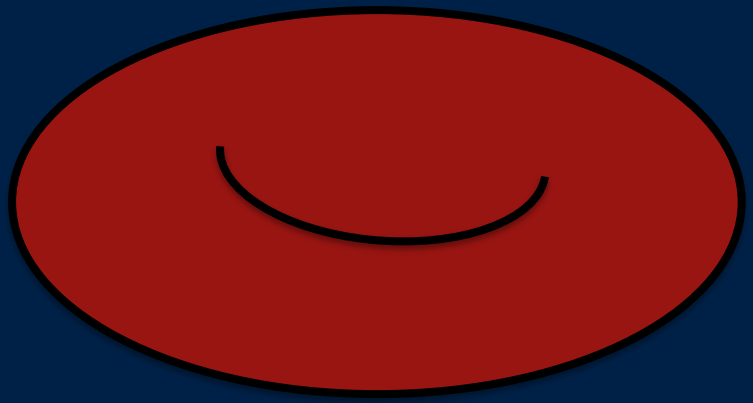


Fine-mapping example 1
Complex genetic variation



Plasmodium falciparum

VS

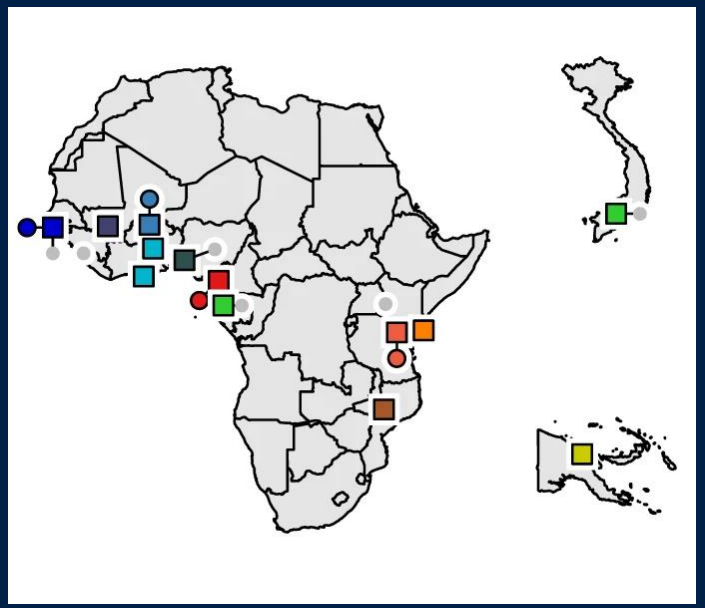


humans

GWAS of susceptibility to severe malaria

Study samples

Group	Cases	Controls	TOTAL
Africa			
Gambia	2567	2605	5172
Mali	274	183	457
Burkina Faso	733	596	1329
Ghana	399	320	719
Nigeria	113	22	135
Cameroon	592	685	1277
Malawi	1182	1317	2499
Tanzania	416	403	819
Kenya	1681	1615	3296
Asia			
Vietnam	718	546	1264
Oceania			
PNG	402	374	776



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Whole–genome sequences

Group	Trios	Duos	Other	TOTAL
Gambia				
FULA	31	1	5	100
JOLA	32	1	2	100
MANDINKA	33	0	1	100
WOLLOF	32	1	3	98
Burkina Faso				
MOSSI	0	0	57	57
Cameroon				
BANTU	5	3	11	31
SEMIBANTU	8	0	7	32
Tanzania				
CHAGGA	21	2	13	80
PARE	22	2	7	77
WASAAMBA	23	6	9	90

GWAS in 17,000 severe malaria cases and population controls
From 12 sites in Africa, Oceania, and SE Asia.
Genotyped on the Illumina Omni 2.5M array

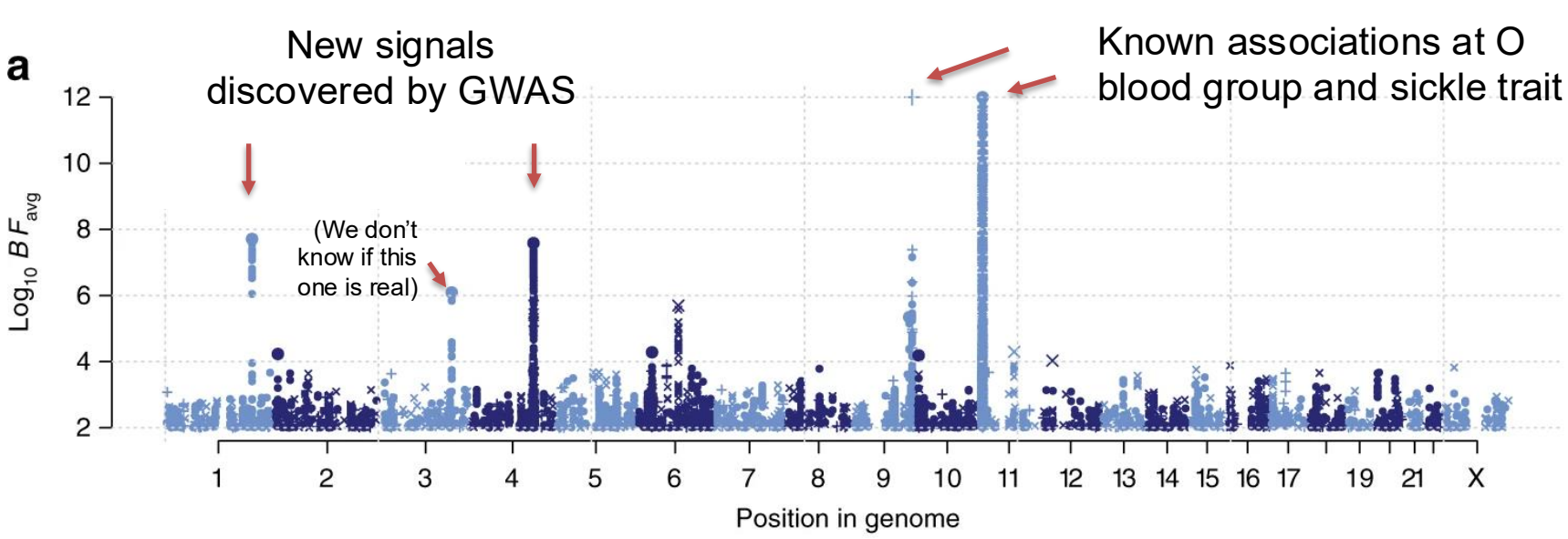
+ whole-genome sequences for imputation

Malaria Genomic Epidemiology Network. *"Insights into malaria susceptibility using genome-wide data on 17,000 individuals from Africa, Asia and Oceania"*.
Nature Communications (2019). https://doi.org/10.1038/s41467-019-13480-z

MalariaGEN
GENOMIC EPIDEMIOLOGY NETWORK

www.malariagen.net

Natural resistance is driven by red blood cell variation



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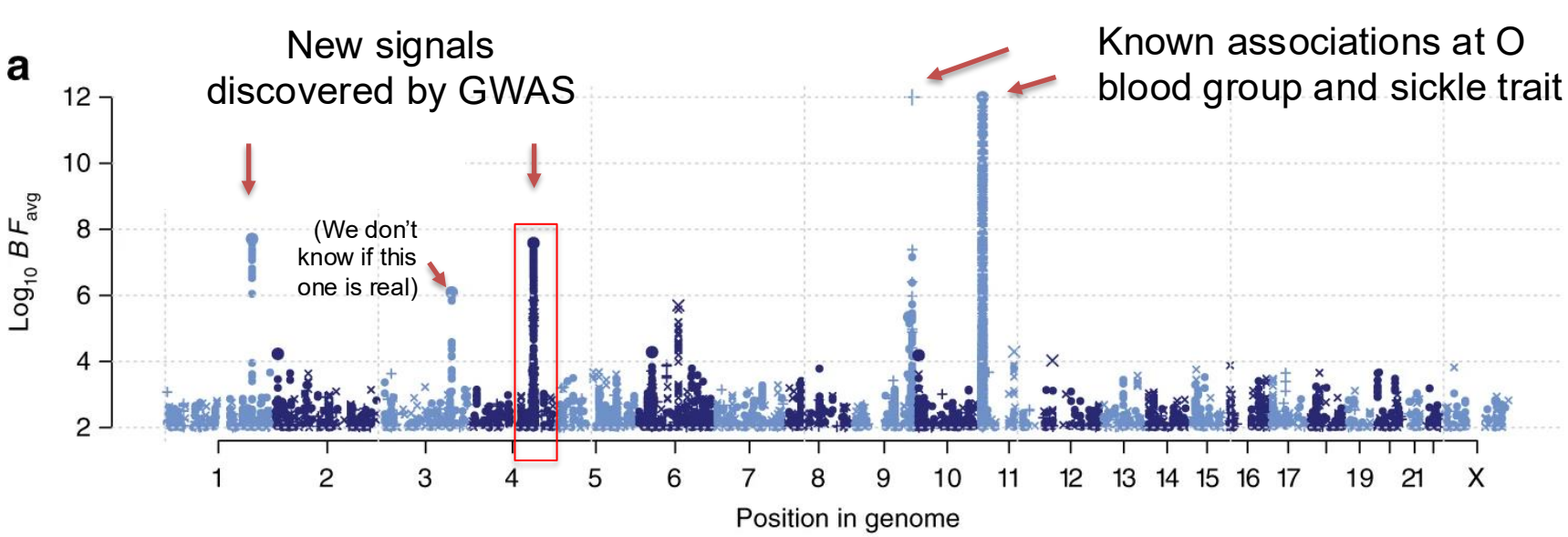
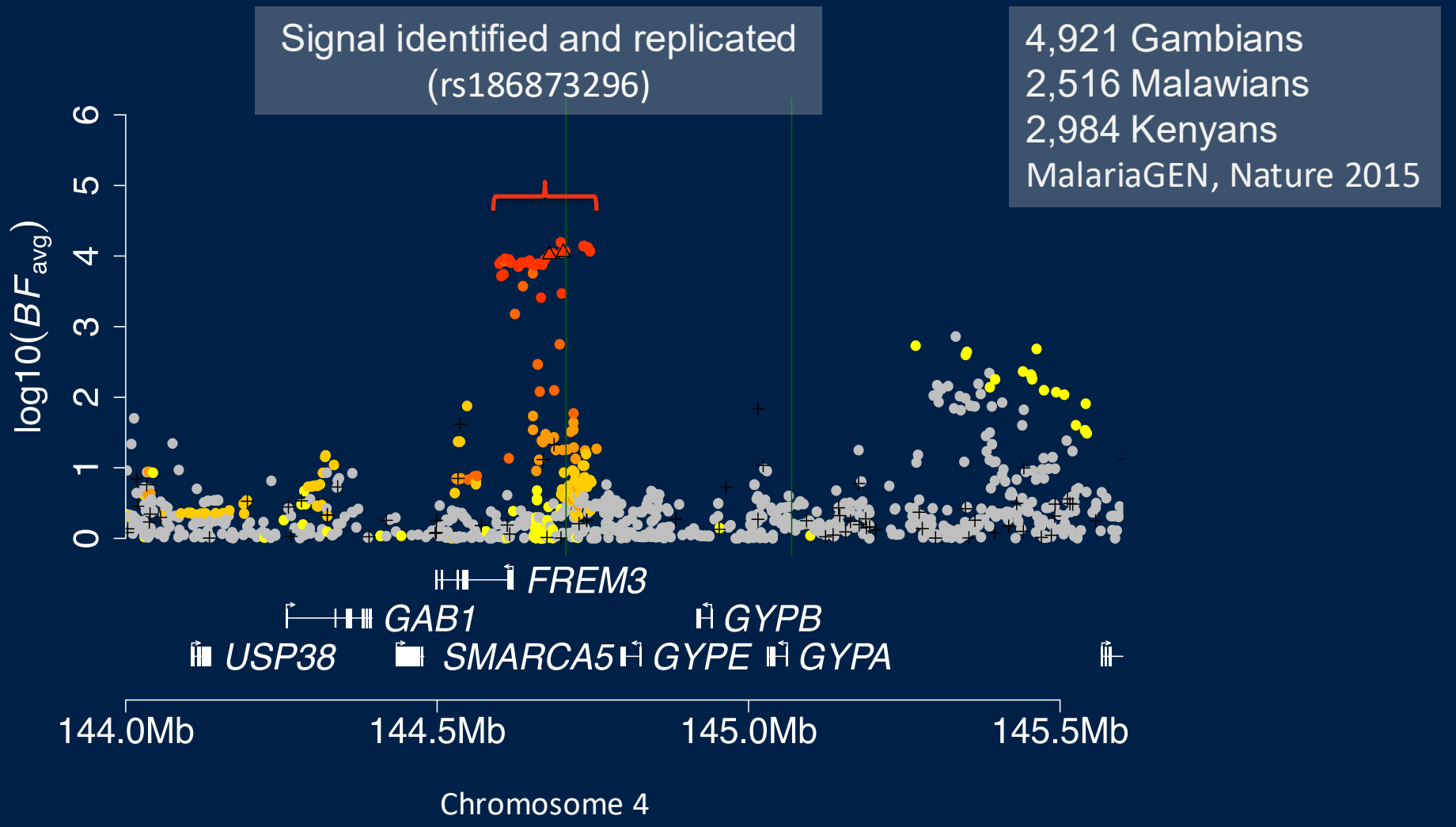
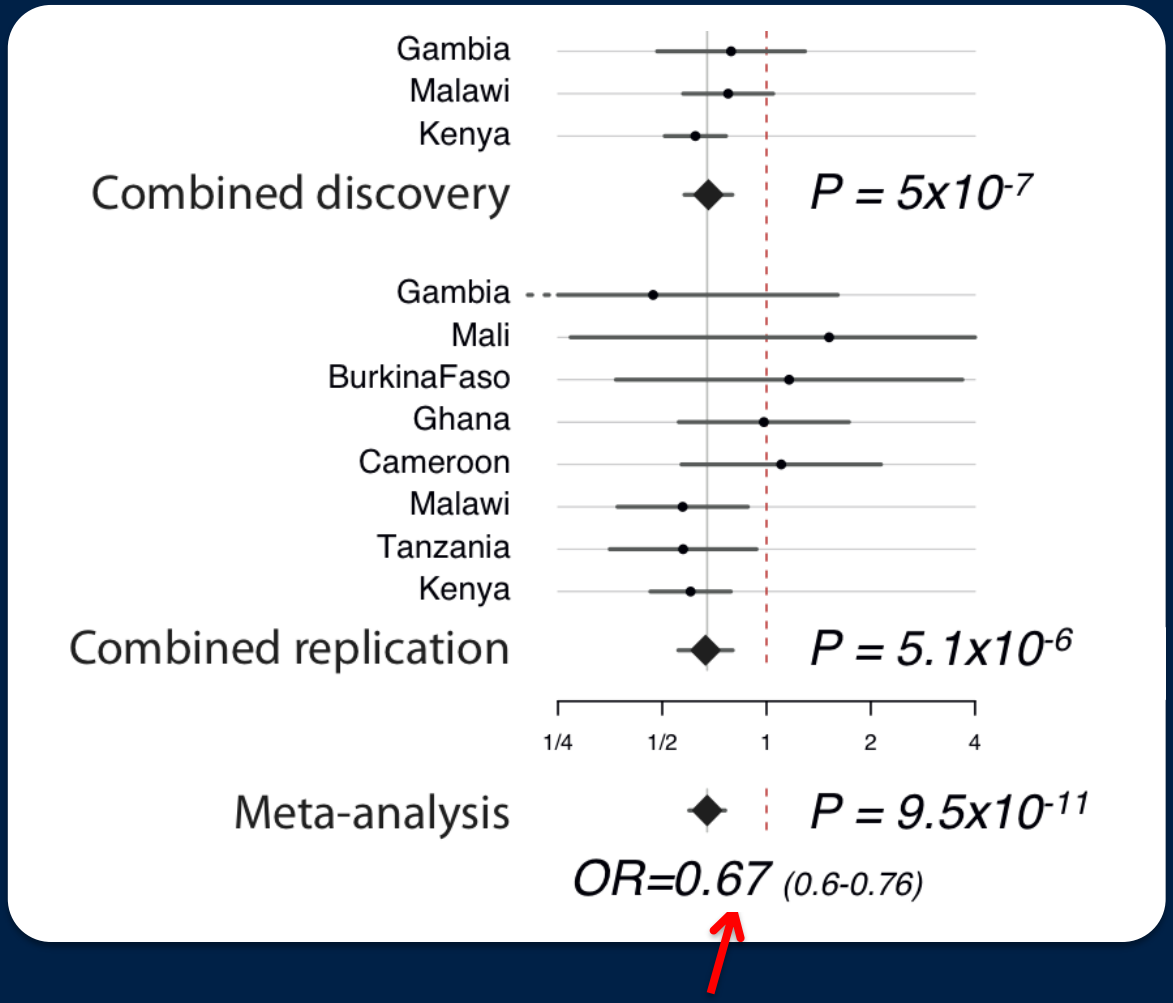


SNPs on chromosome 4 are associated with proection against severe malaria



The association has quite large effect



> 30% protective effect per copy of the derived allele

$$\text{Standard error}(\log OR) \approx \frac{1}{\sqrt{N \times f(1-f) \times \phi(1-\phi)}}$$

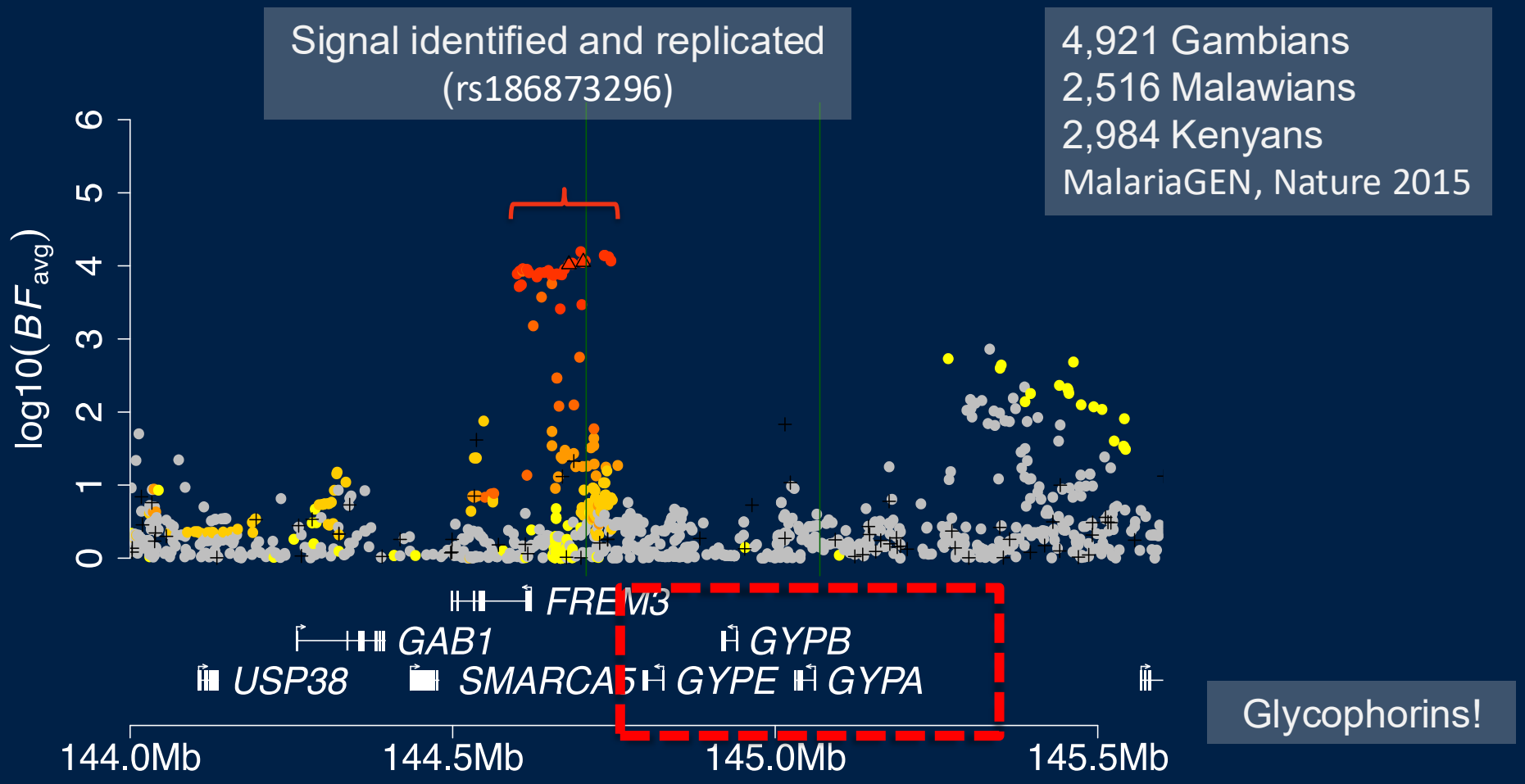
Can we finemap?

We had an exciting association. But fine-mapping has proven to be difficult for many GWAS loci.

To hope for success we might need:

- Good candidates for the functional gene?
- Good candidates for the causal mutation(s)?

SNPs on chromosome 4 are associated with proection against severe malaria



Glycophorins encode the ‘MNS’ blood group

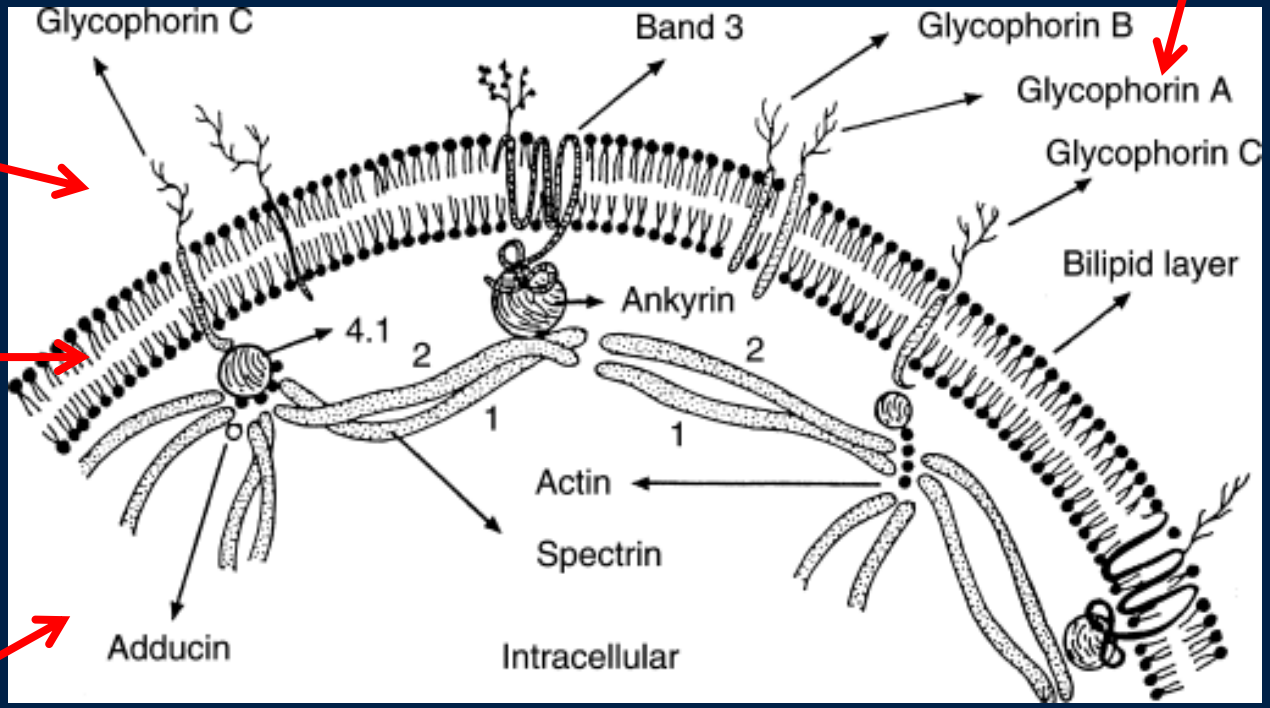
(antigenic molecules on RBC surface)

Glycophorins

Outside red blood cell

Red blood cell membrane

Inside red blood cell

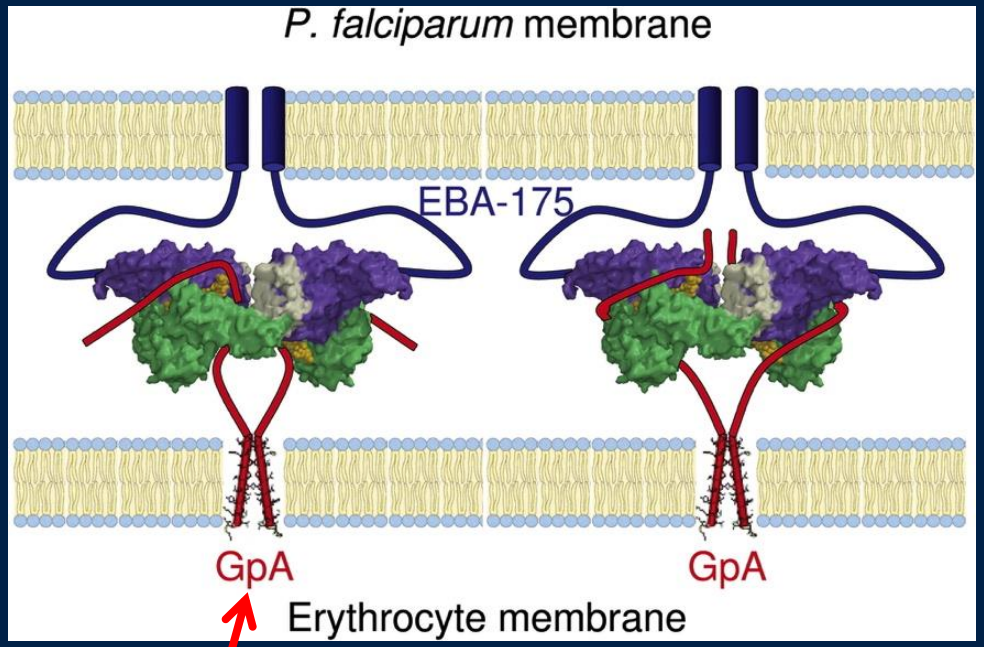


Grimes and Slater, The Inherited Metabolic Diseases, 1994

Glycophorins are receptors for *P.falciparum* during red blood cell invasion

P. Falciparum parasite

red blood cell



Glycophorin A

Miller et al, J. Exp. Med 1979

Tolia et al, Cell 2005

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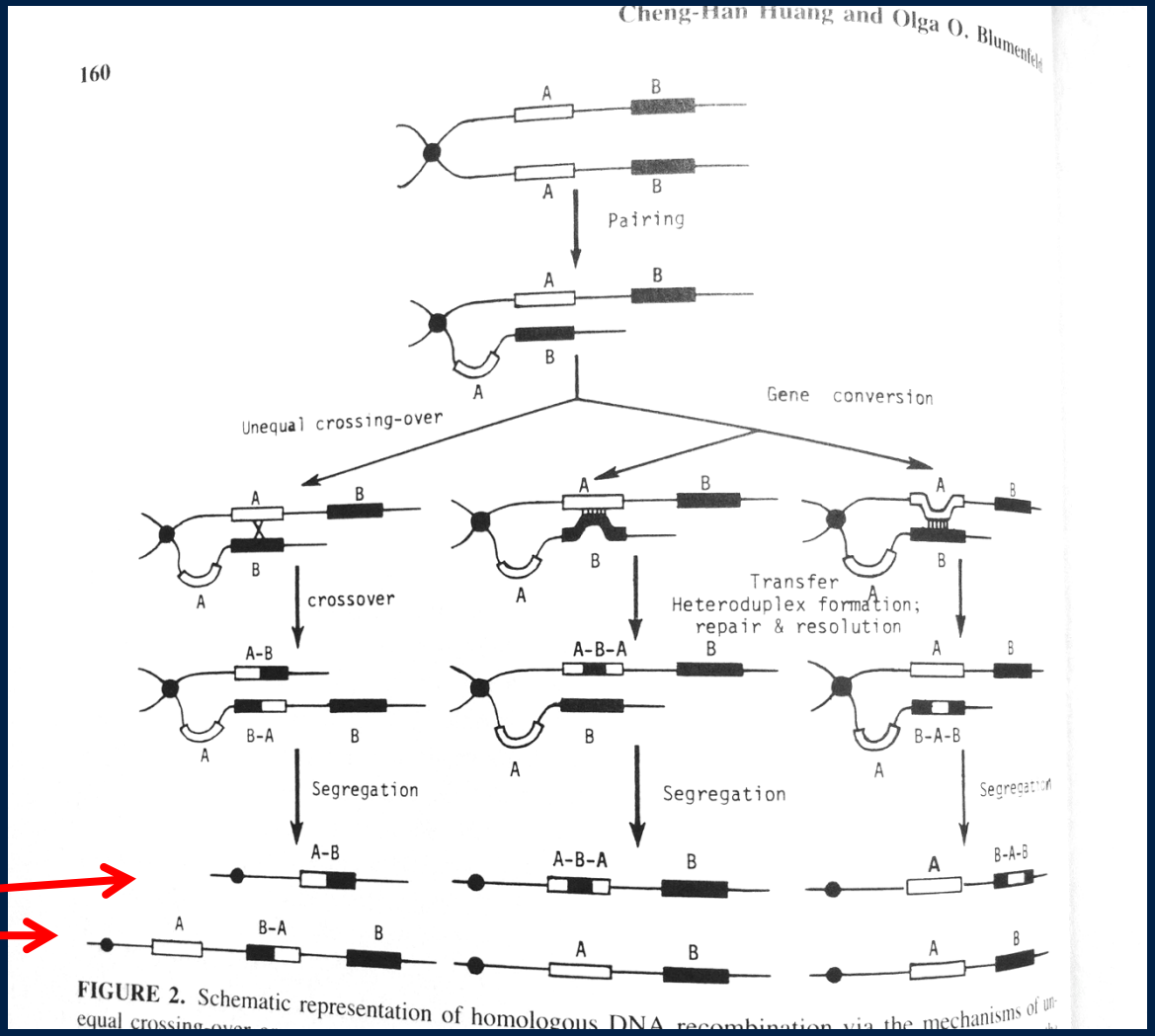
- Good candidates for the causal mutation(s)?

Structural variants create deletions, duplications, and hybrid genes

The MNS blood group is highly diverse, with over 45 known antigens.

Encoded by single nucleotide polymorphisms and structural variants

Deleted / duplicated / hybrid genes



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- ✅ - Good candidates for the causal mutation(s)?

Steps to fine-map

Step 1: type or sequence as much of the genetic variation in the region as possible – hope to catch the causal mutation.

Step 2: re-analyse the association.

Step 3: look for functional mutations

A regional reference panel capturing structural variation

We used the >3,600 samples including
- 1000 Genomes Project Phase III reference panel
- plus our newly-sequenced samples

a

Whole–genome sequences

group	trios	duos	other	TOTAL
Gambia				
FULA	31	1	5	**100**
JOLA	32	1	2	**100**
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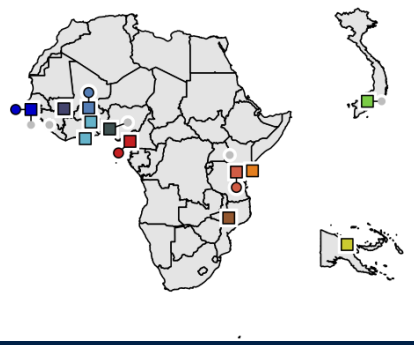
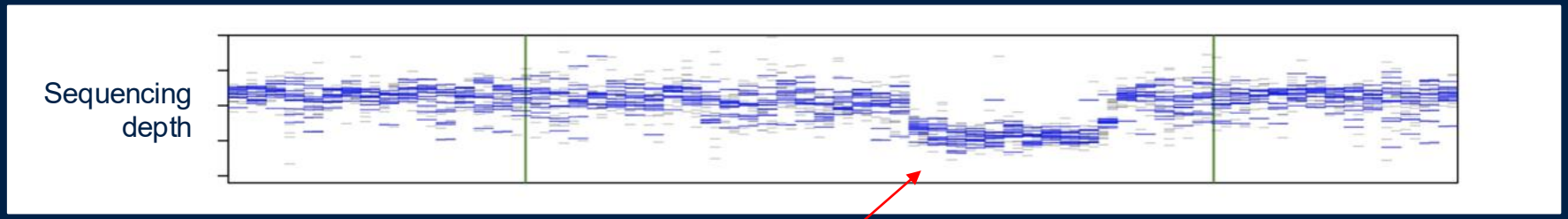
Burkina Faso				
MOSSI	0	0	57	**57**
Cameroon				
BANTU	5	3	11	**31**
SEMIBANTU	8	0	7	**32**
Tanzania				
CHAGGA	21	2	13	**80**
PARE	22	2	7	**77**
WASAAMBA	23	6	9	**90**



…to call SNPs and indels and structural variation.

Illustration of structural variant calling:



(this sample has a deletion in this region)

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a

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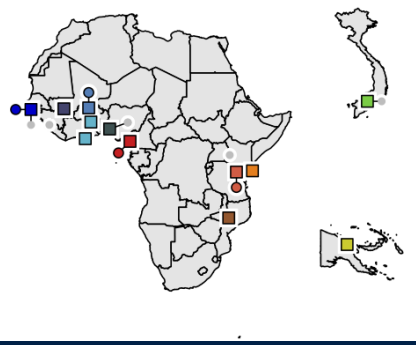
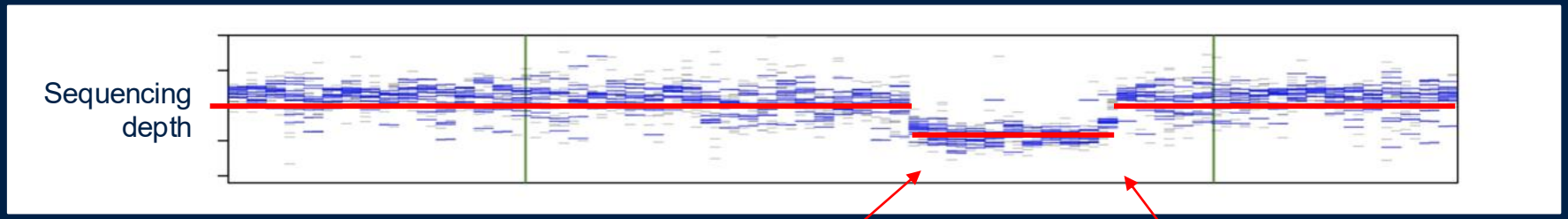
WOLLOF	32	1	3	**98**
Burkina Faso				
MOSSI	0	0	57	**57**
Cameroon				
BANTU	5	3	11	**31**
SEMIBANTU	8	0	7	**32**
Tanzania				
CHAGGA	21	2	13	**80**
PARE	22	2	7	**77**
WASAAMBA	23	6	9	**90**



…to call SNPs and indels and structural variation.

Illustration of structural variant calling:



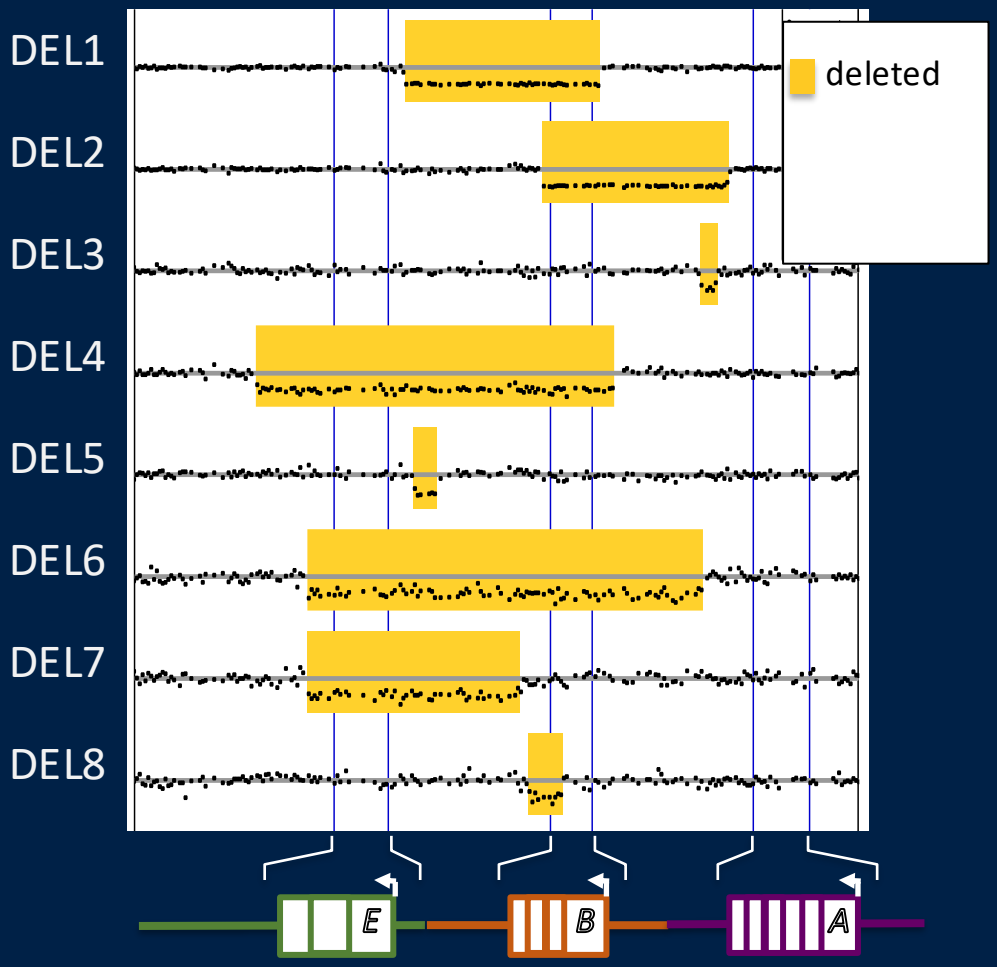
(this sample has a deletion in this region)

…our method infers the copy number

The region turned out to have *a lot* of structural variation

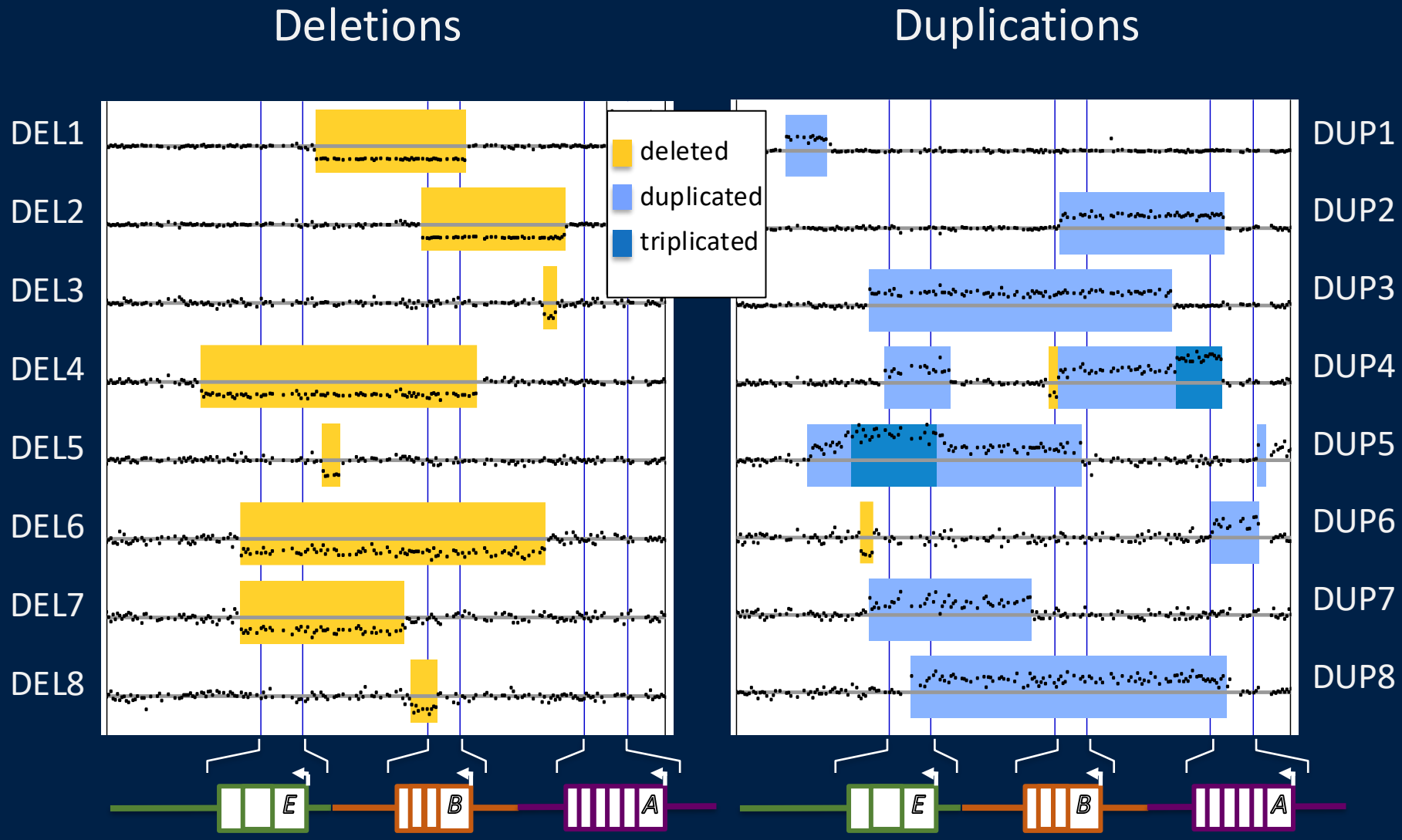
Deletions

Duplications



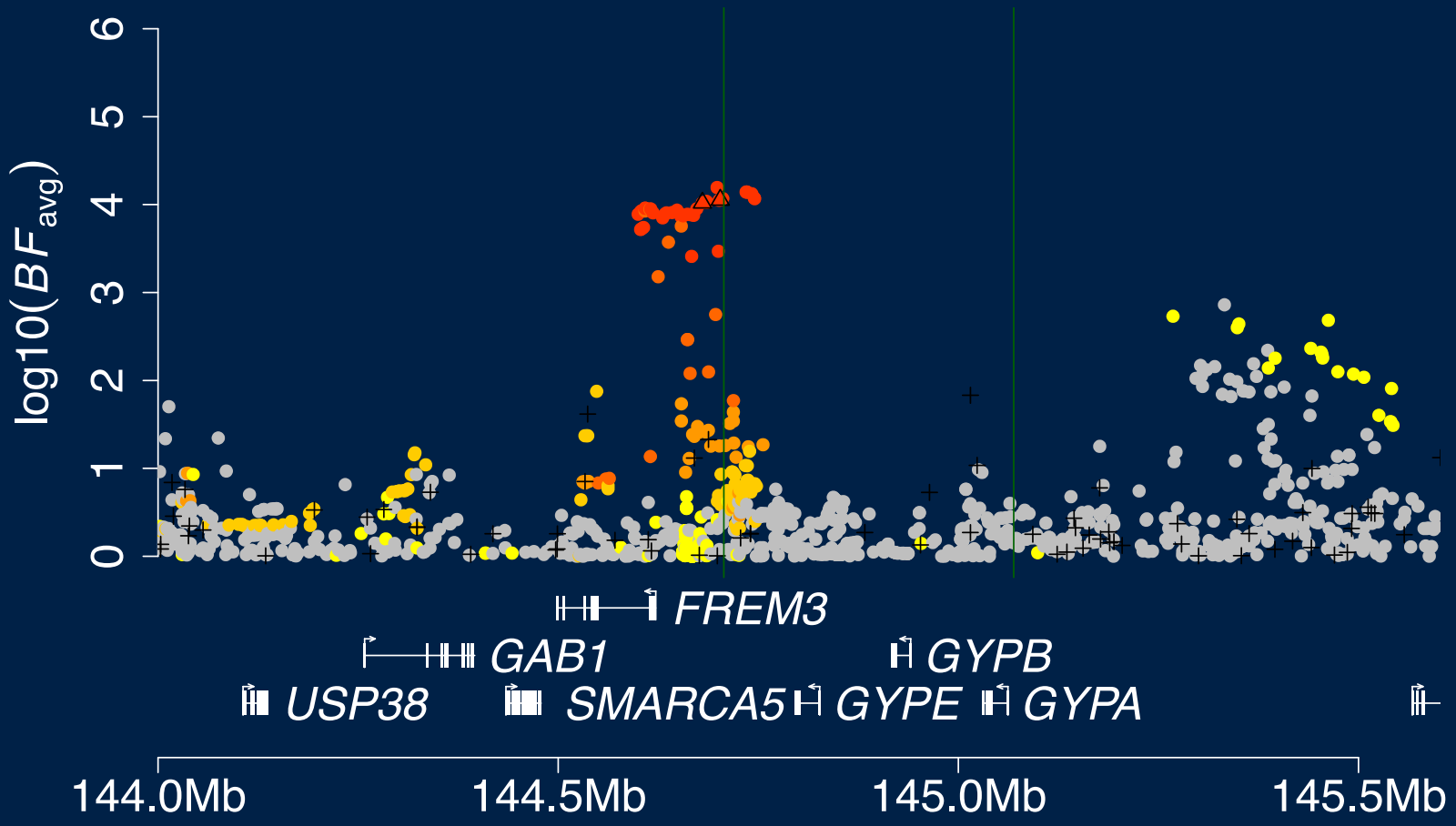
14% of Africans carry a CNV affecting these genes

The region turned out to have *a lot* of structural variation



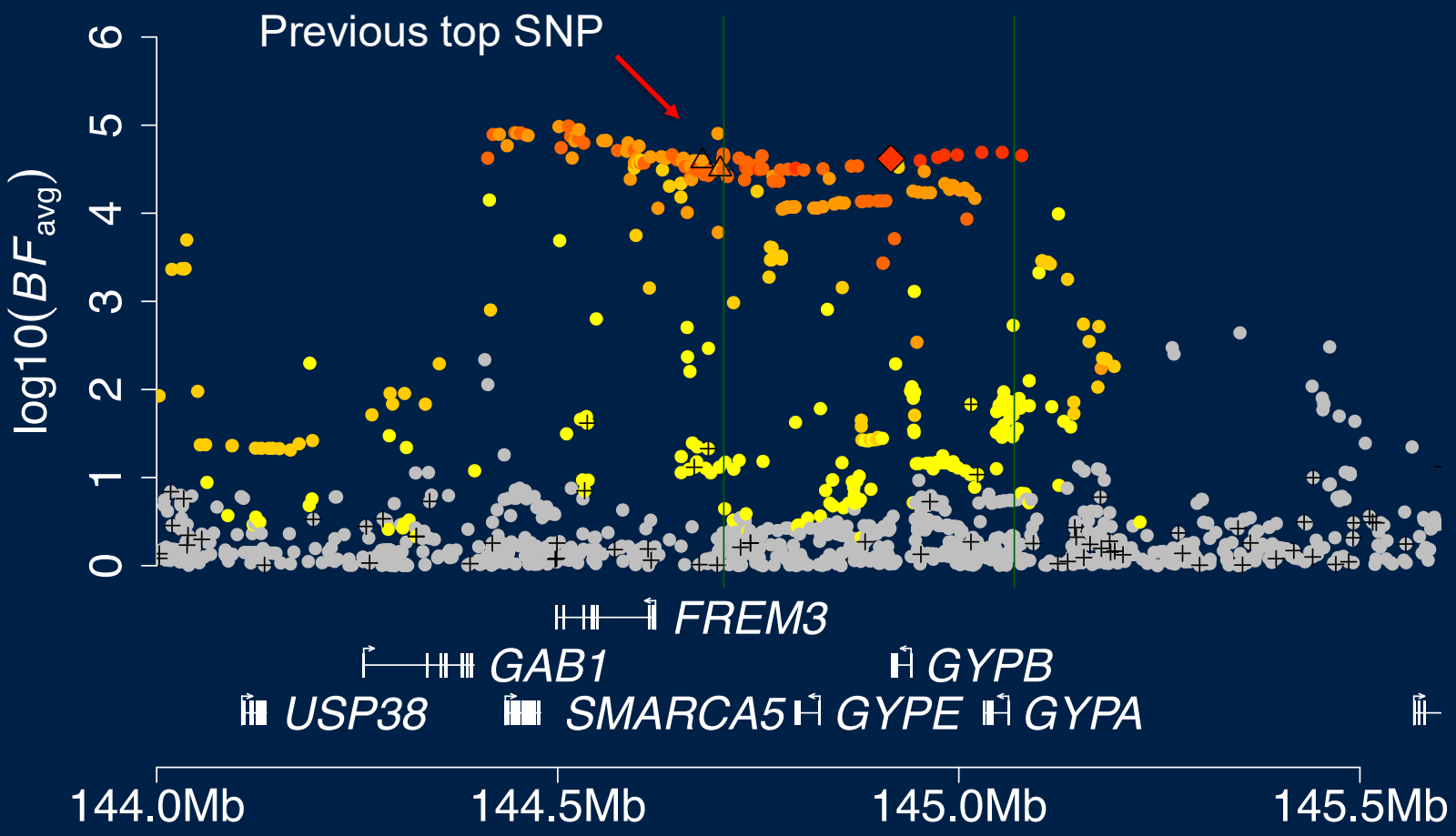
14% of Africans carry a CNV affecting these genes

Before fine-mapping



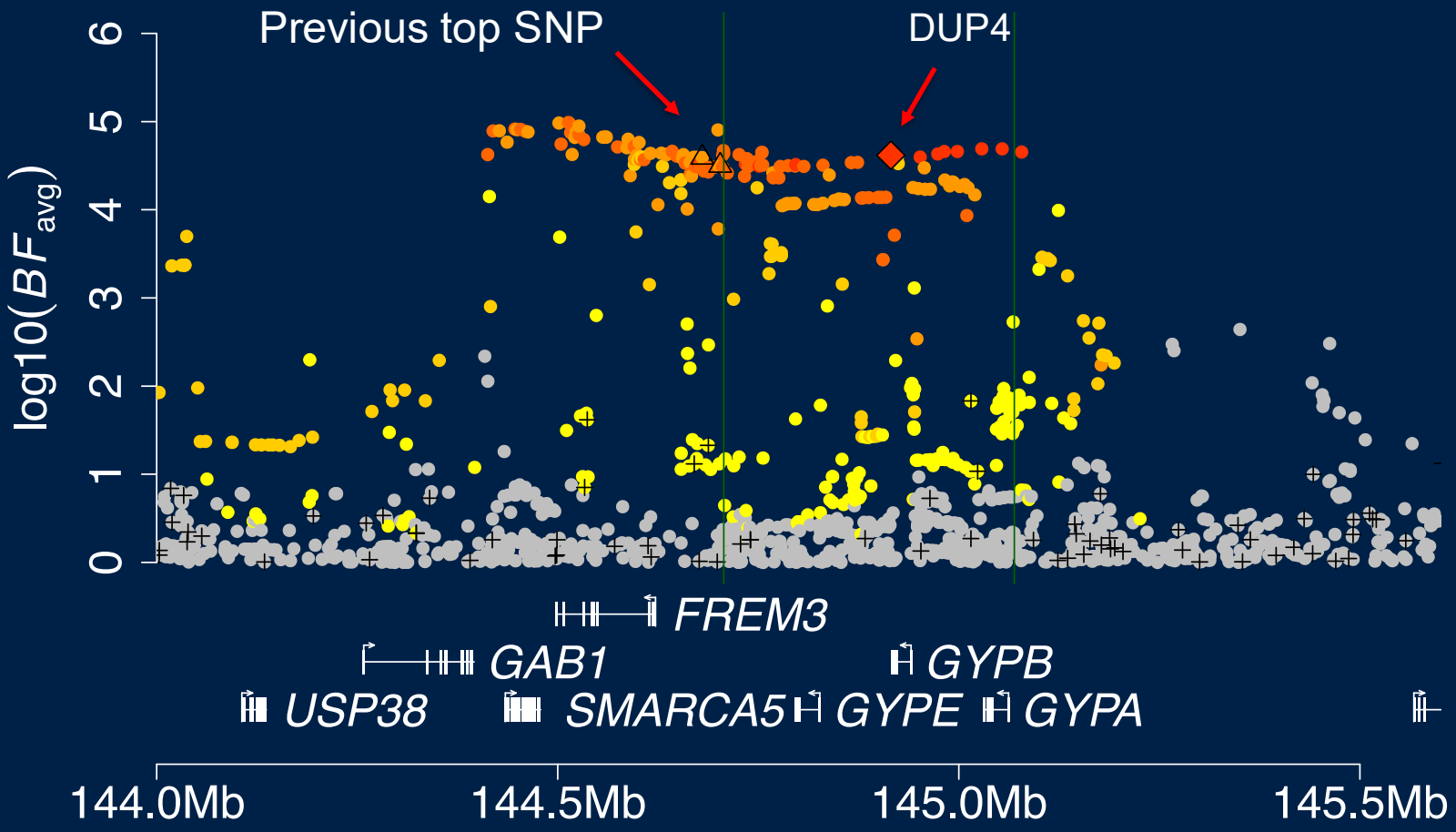
Original GWAS result

After fine-mapping

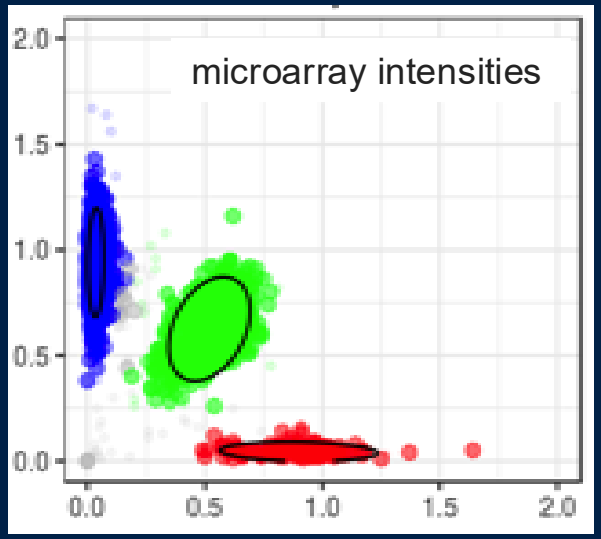


Result after incorporating genetic variation discovered in sequenced samples.

After fine-mapping



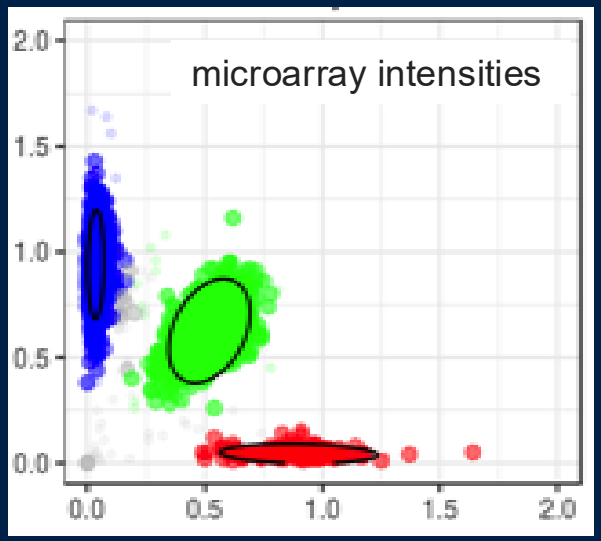
Confirming structural variants using cluster plots



This is how a microarray cluster plot should look: 3 clusters for AA / AB / BB genotypes

Confirming structural variants using cluster plots

Actually this signal was evident in our cluster plots



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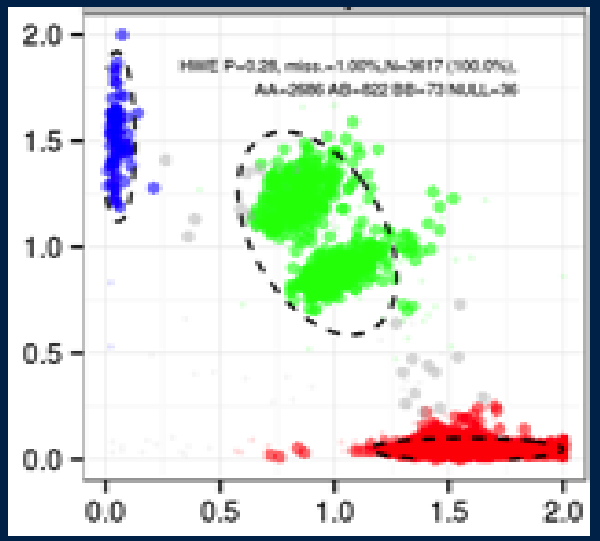
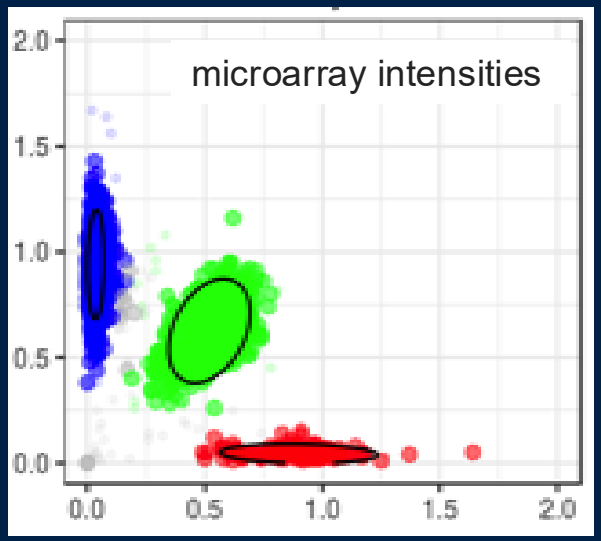


What we saw in this region

Confirming structural variants using cluster plots

Still true that nothing seemed to be functional. What next?



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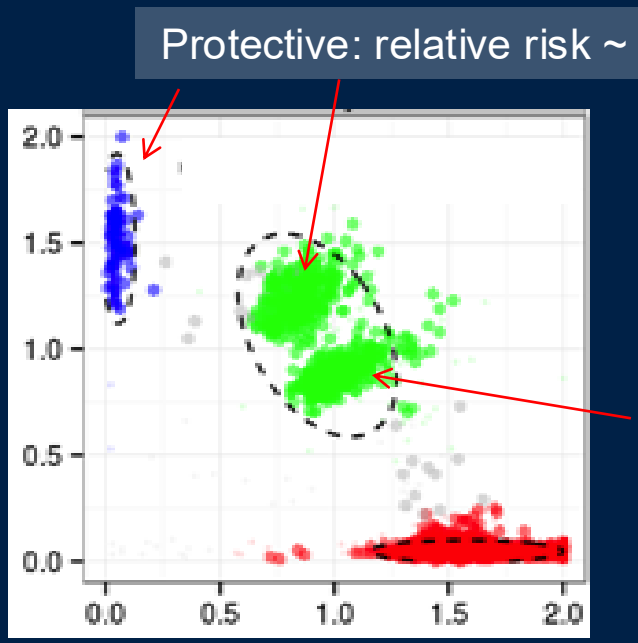
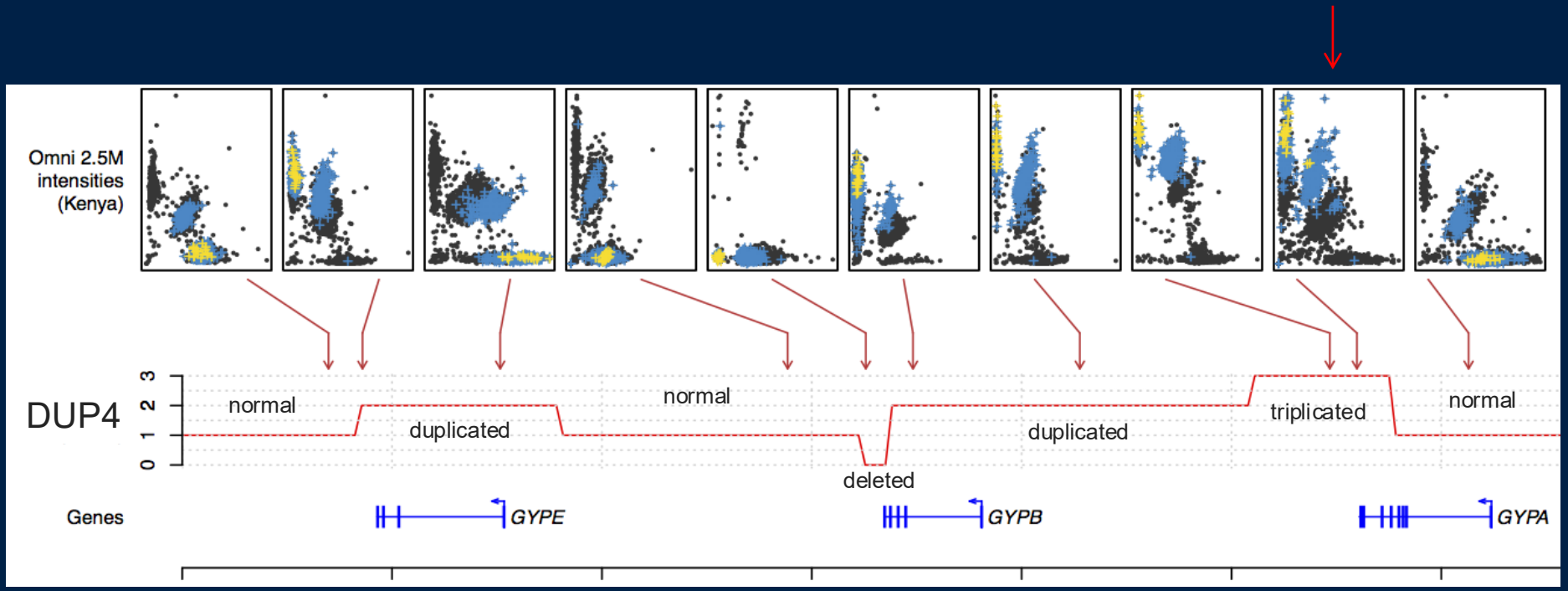


What we saw in this region

Confirming structural variants using cluster plots

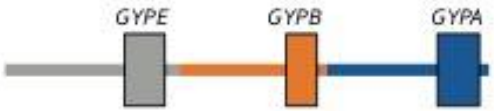


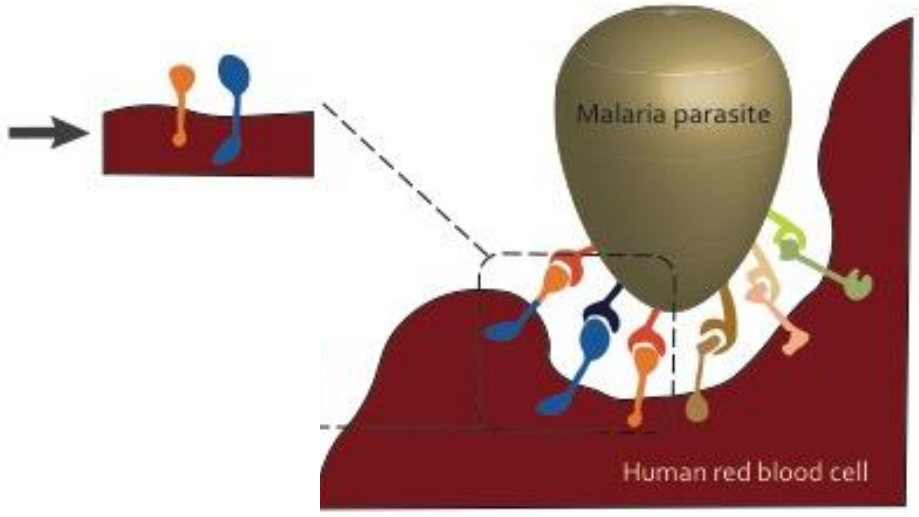
We were able to use cluster plots to confirm individuals in our GWAS really do carry the complicated structural variant “DUP4”.

DUP4 is pretty complicated – what could it be?

What is DUP4?

"Normal" haplotype:

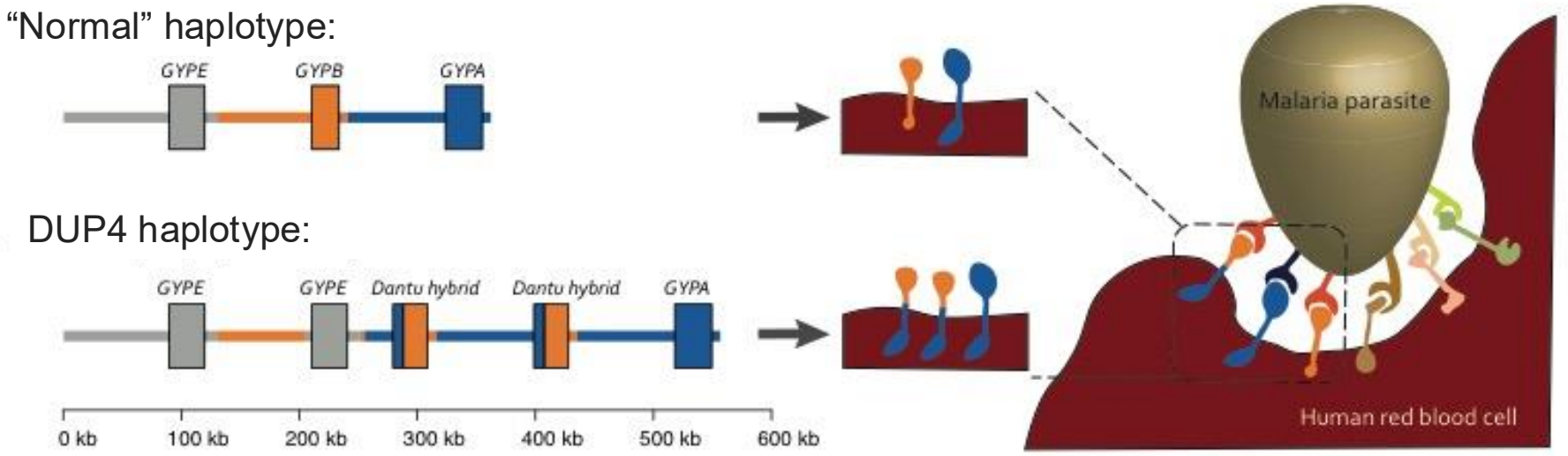




Leffler et al, "*Resistance to malaria through structural variation of red blood cell invasion receptors*", Science (2017)

https://doi.org/10.1126/science.aam6393

What is DUP4?



Leffler et al, "*Resistance to malaria through structural variation of red blood cell invasion receptors*", Science (2017)

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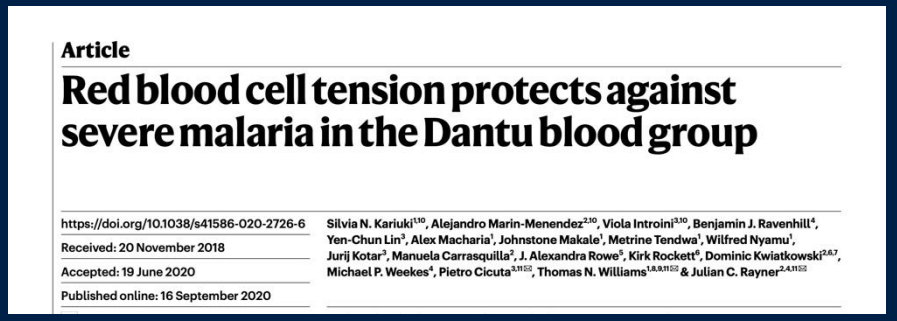
Functional followup study



Article

Red blood cell tension protects against severe malaria in the Dantu blood group

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Silvia N. Kariuki[1,10], Alejandro Marin-Menendez[2,10], Viola Introini[3,10], Benjamin J. Ravenhill[4], Yen-Chun Lin[3], Alex Macharia[1], Johnstone Makale[1], Metrine Tendwa[1], Wilfred Nyamu[1], Jurij Kotar[3], Manuela Carrasquilla[2], J. Alexandra Rowe[5], Kirk Rockett[6], Dominic Kwiatkowski[2,6,7], Michael P. Weekes[4], Pietro Cicuta[3]✉, Thomas N. Williams[1,8,9]✉ & Julian C. Rayner[2,4]✉

https://doi.org/10.1038/s41586-020-2726-6

Dantu is globally rare...

The Dantu blood group has been found in:

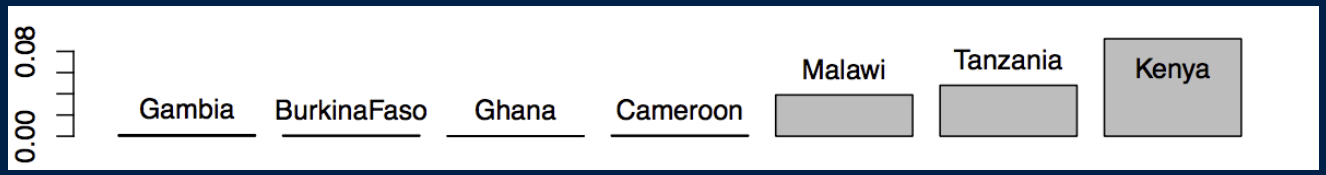
1 in **44,112**	Londoners*
0 in **1,000**	Germans†
1 in **320**	African Americans†
0 in **2870**	Gambians‡

…but found at high frequency in east Africa

The Dantu blood group has been found in:

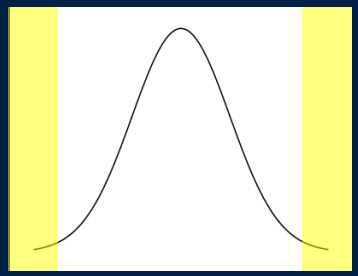
1 in **44,112**	Londoners*
0 in **1,000**	Germans†
1 in **320**	African Americans†
0 in **2870**	Gambians‡
1 in **12**	Malawians‡
1 in **6**	Kenyans (from the Kilifi region)‡

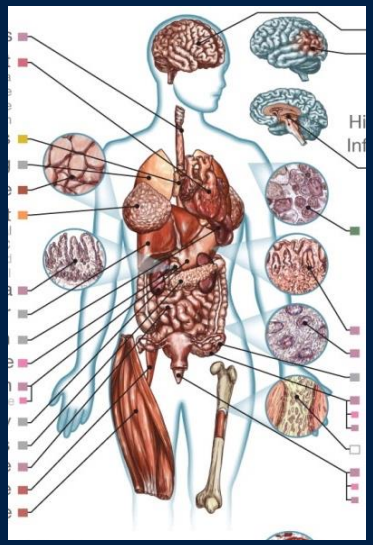
Allele frequency:



West Africa ⟷ East Africa

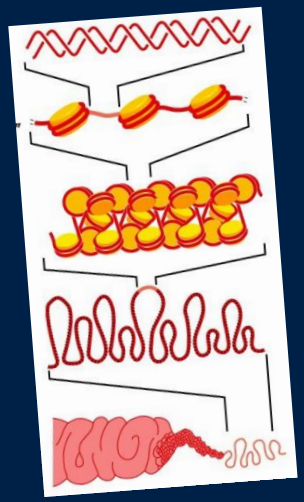
The circle of genetic causation

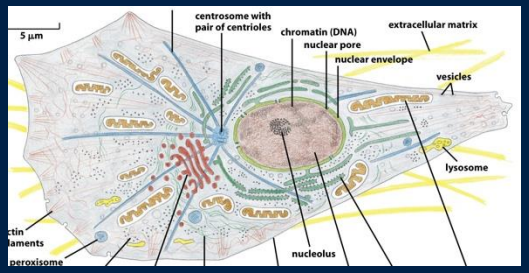




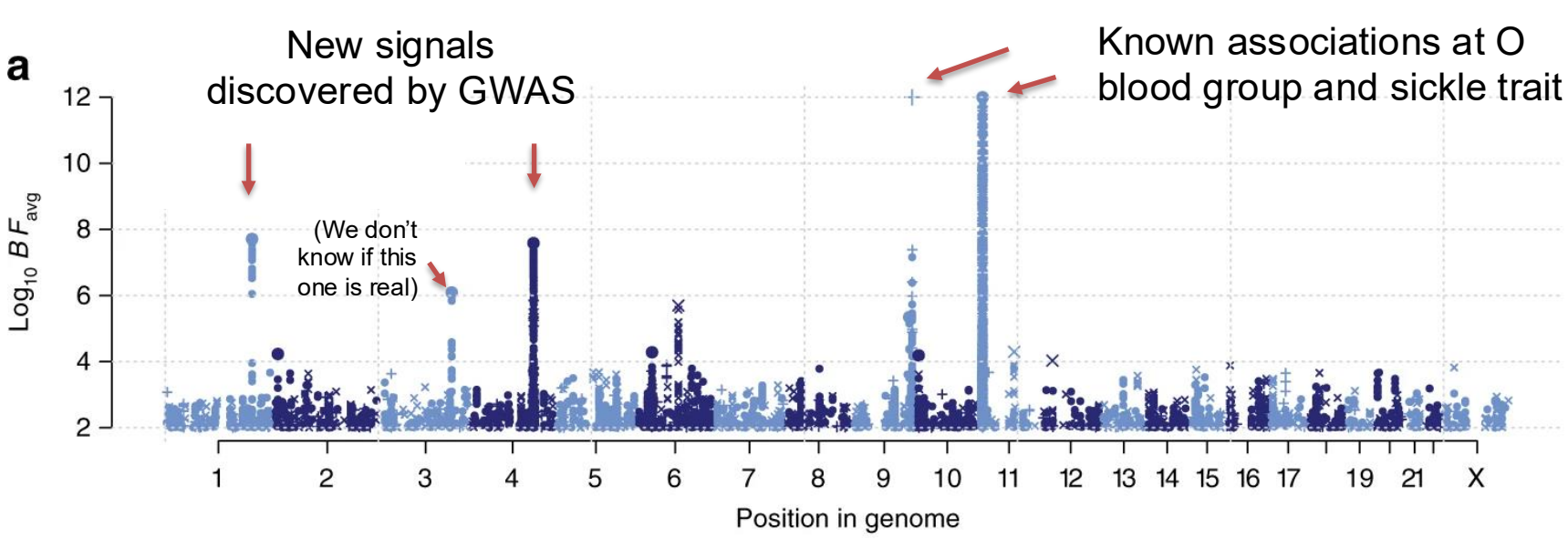
...that combine to make individuals...

Fine-mapping example 2
Cell-specific gene regulation





Natural resistance is driven by red blood cell variation



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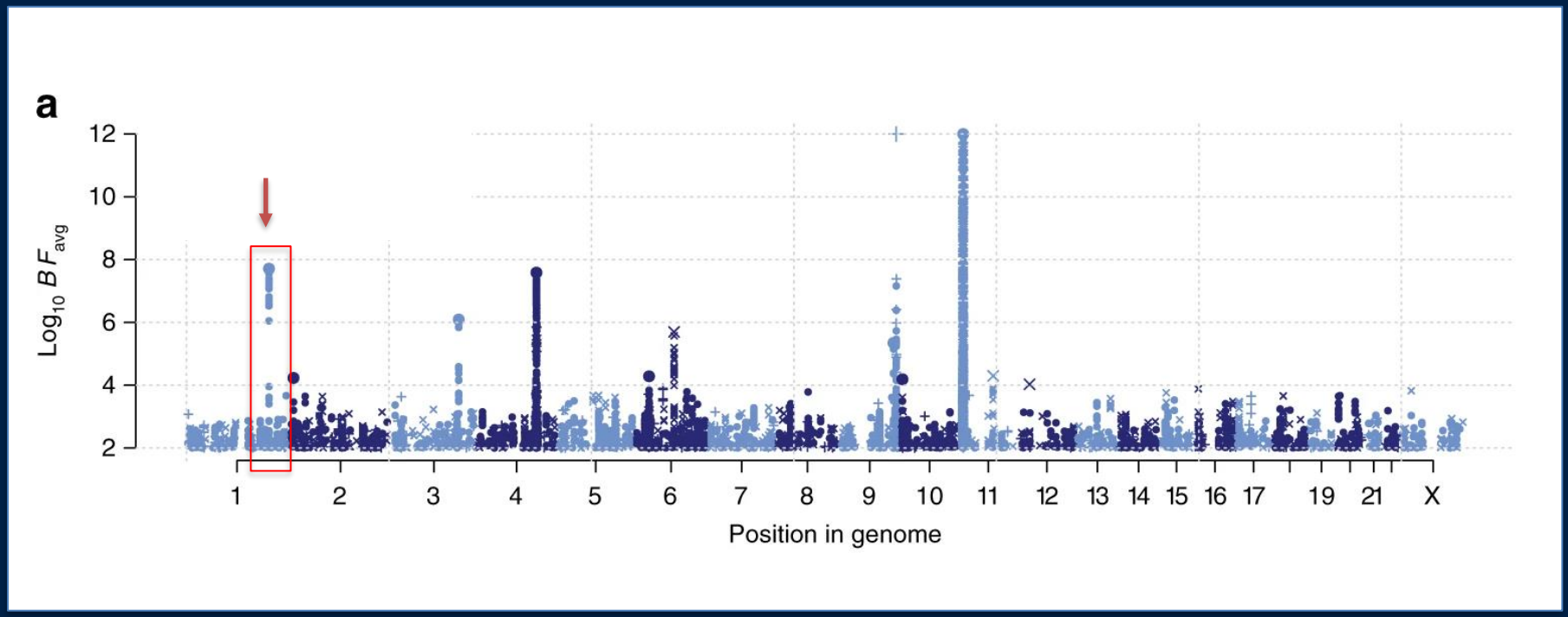
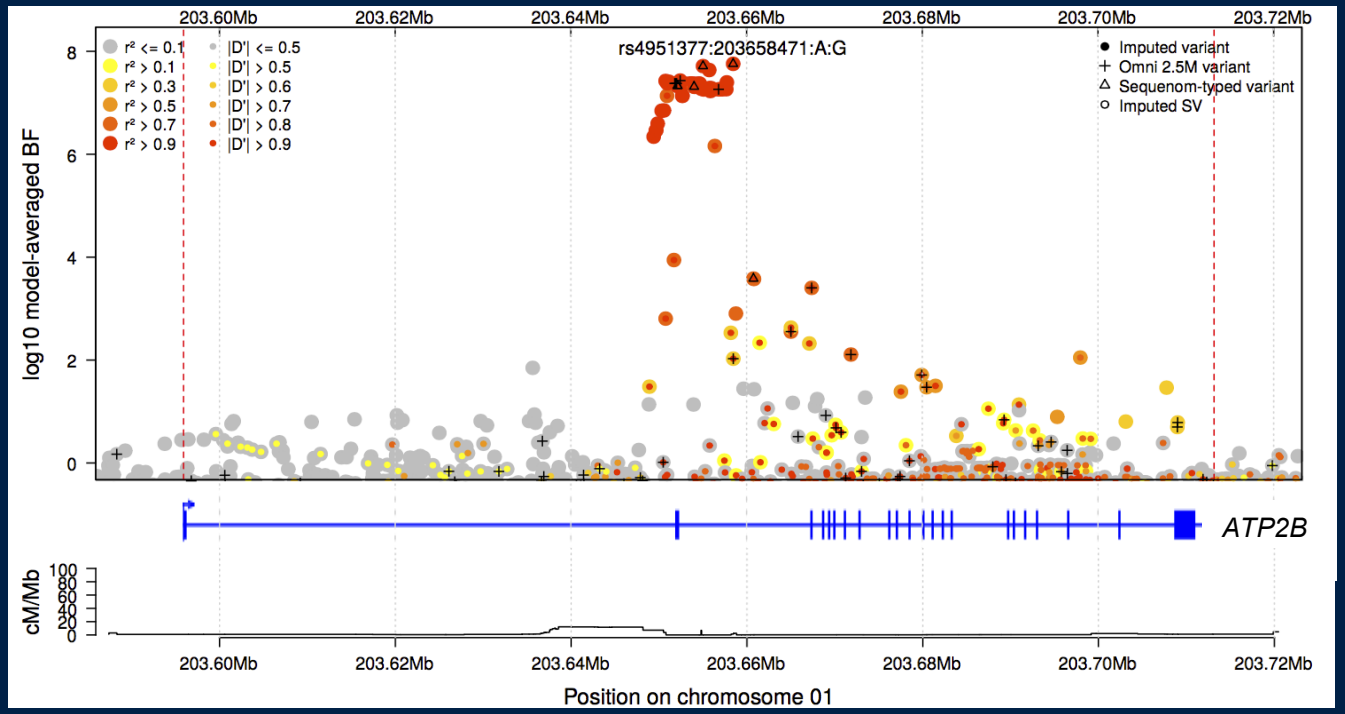


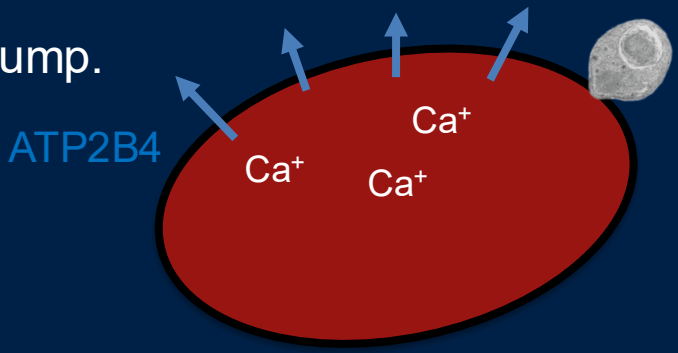
Genome-wide association study of severe malaria susceptibility

Association near 2nd exon of *ATP2B4*

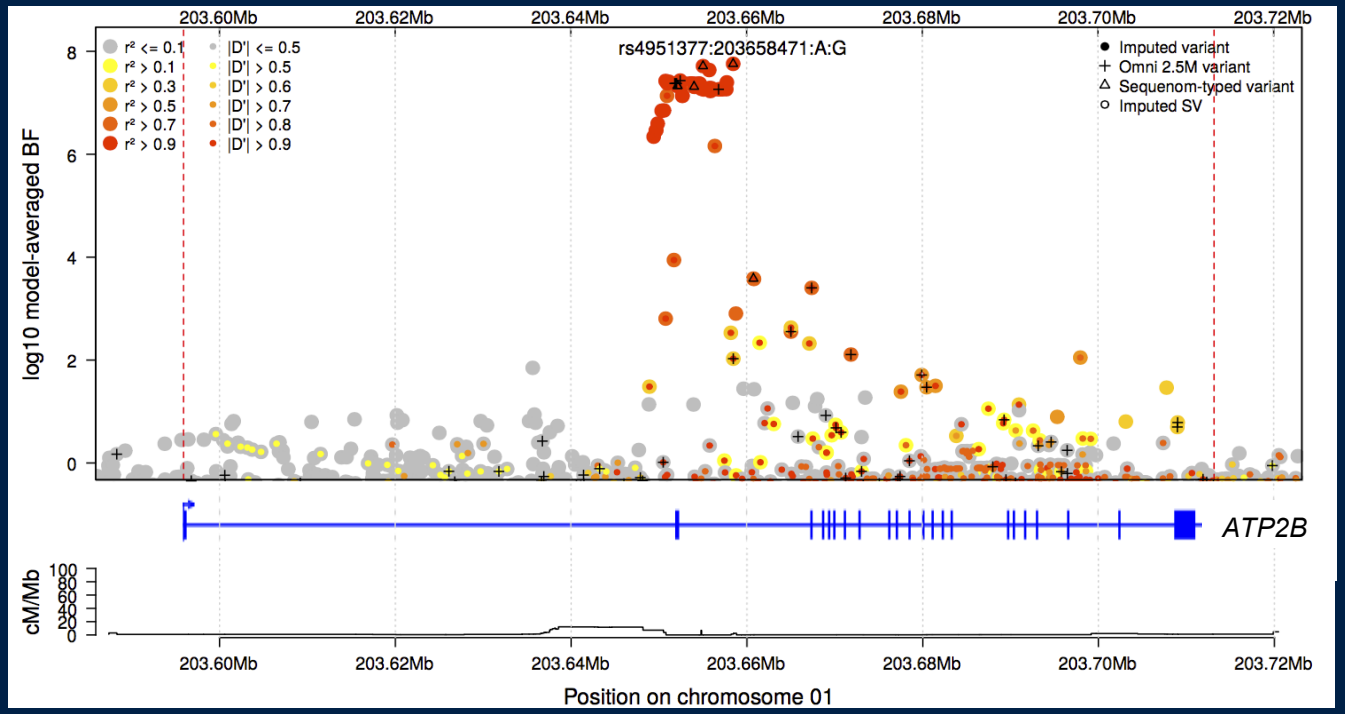


The associated SNPs cover a region around the second exon.

In a gene called *ATP2B4,* which encodes a calcium pump.



Association near 2nd exon of *ATP2B4*

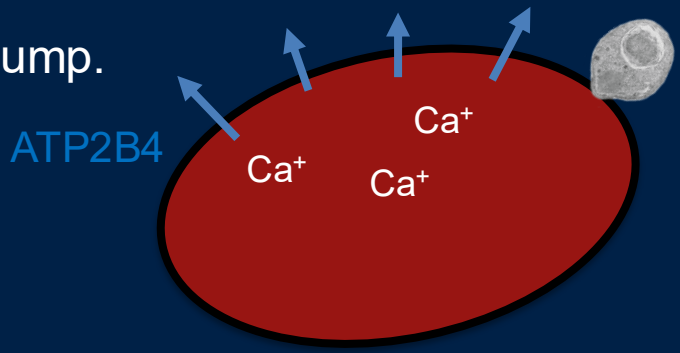


The associated SNPs cover a region around the second exon.

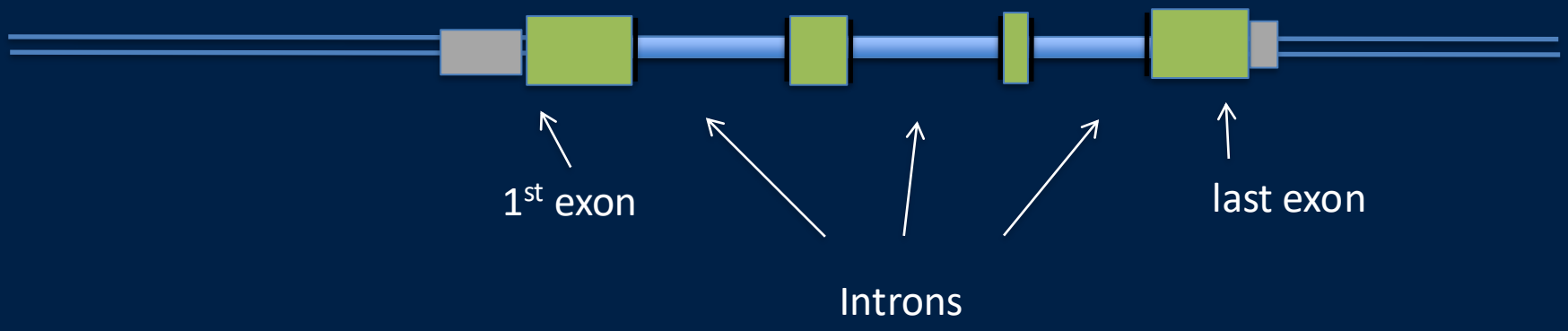
In a gene called *ATP2B4,* which encodes a calcium pump.

None of these SNPs make changes to the protein.

What could be going on?



Cartoon of a gene



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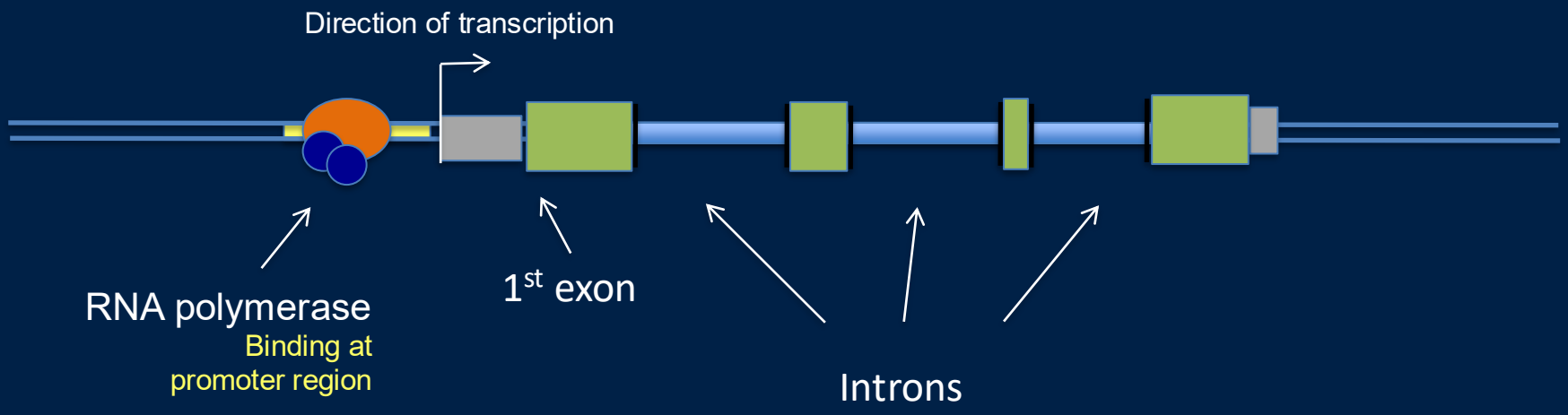

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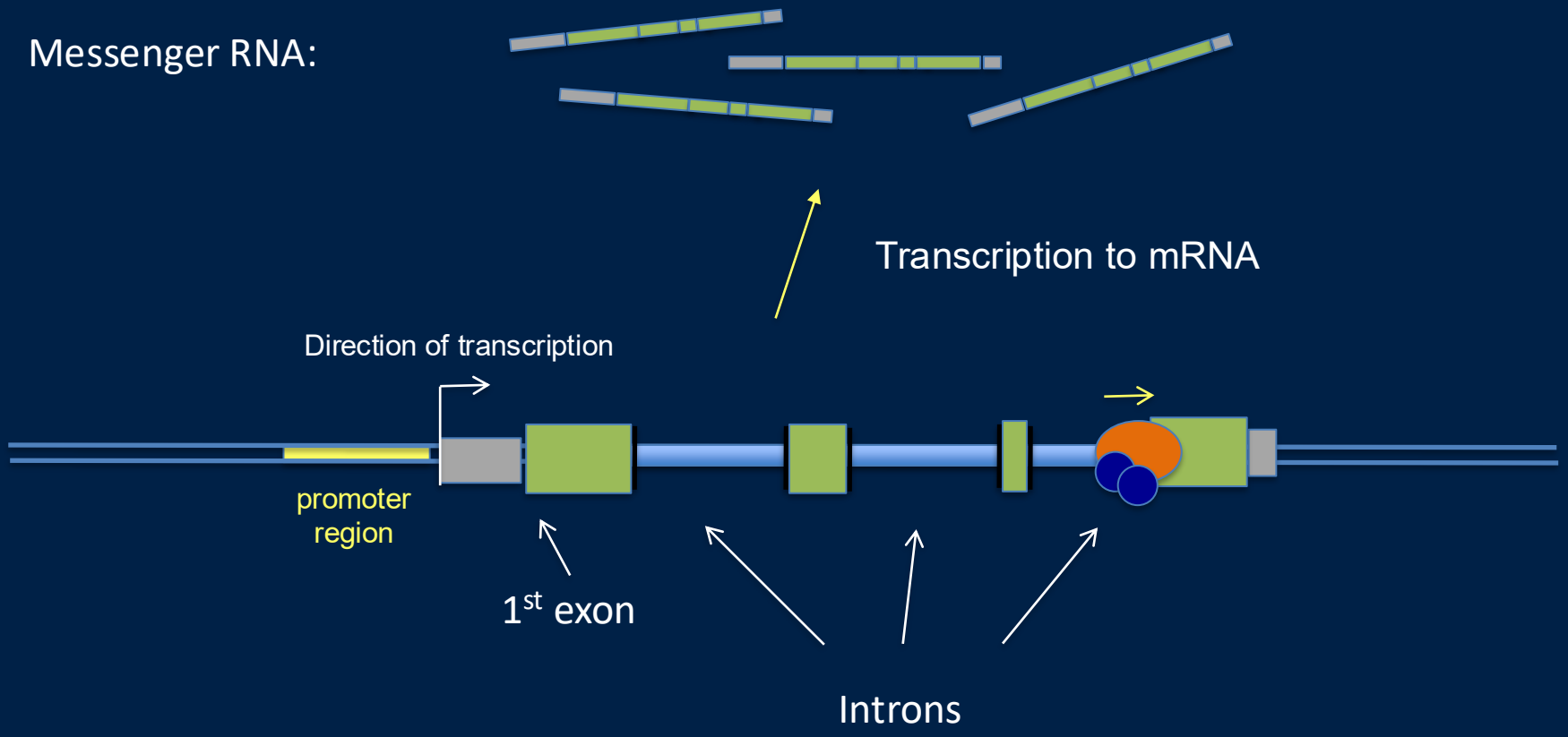
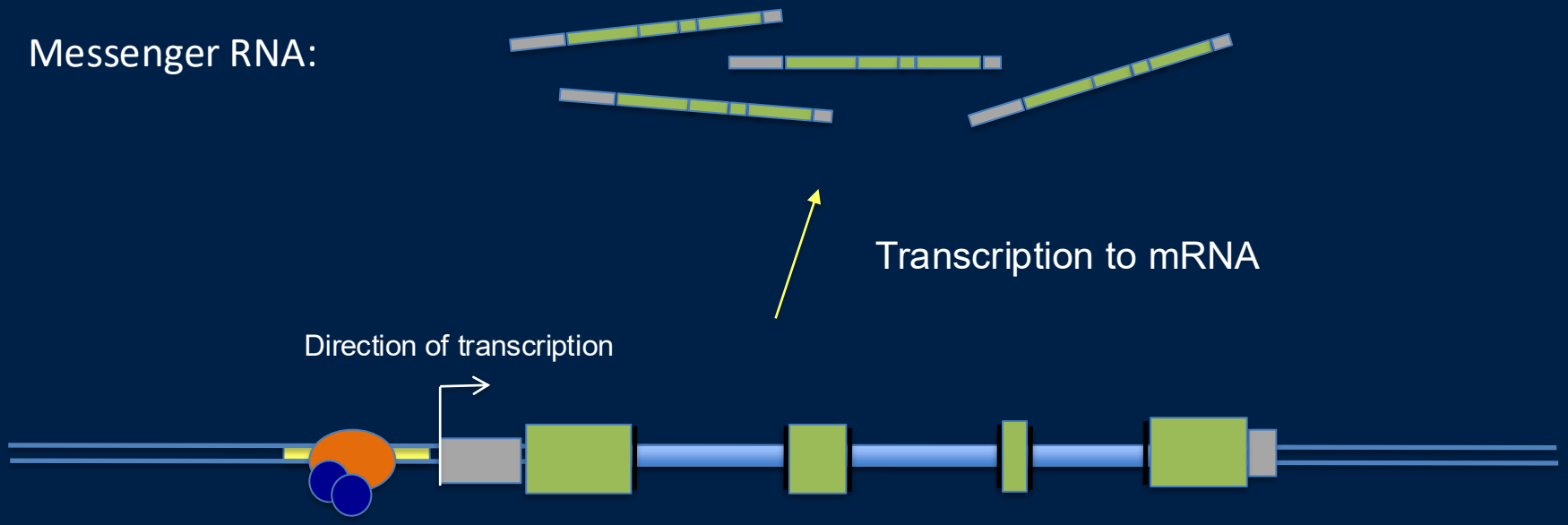


Cartoon of a gene



What happens at the promoter can really affect transcription.

Cartoon of a gene

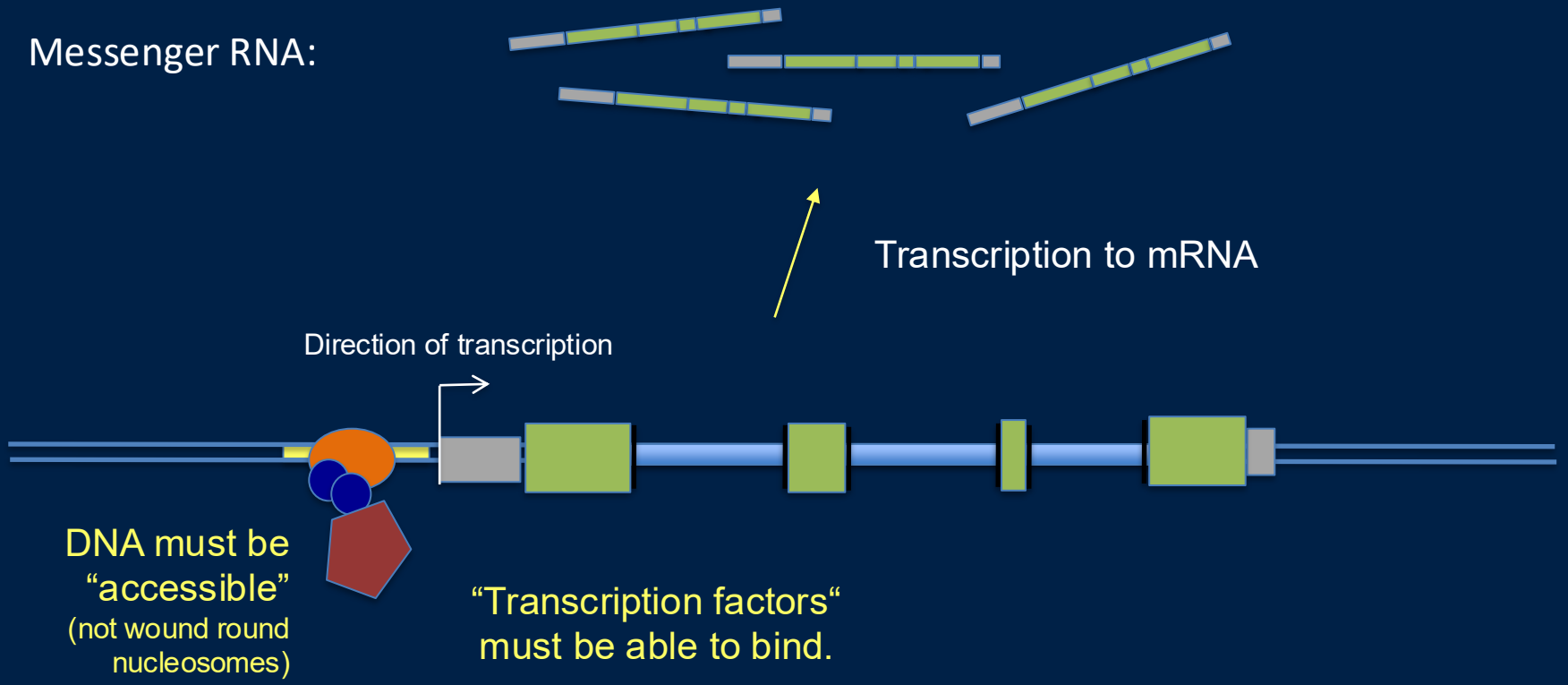
Messenger RNA:

Transcription to mRNA

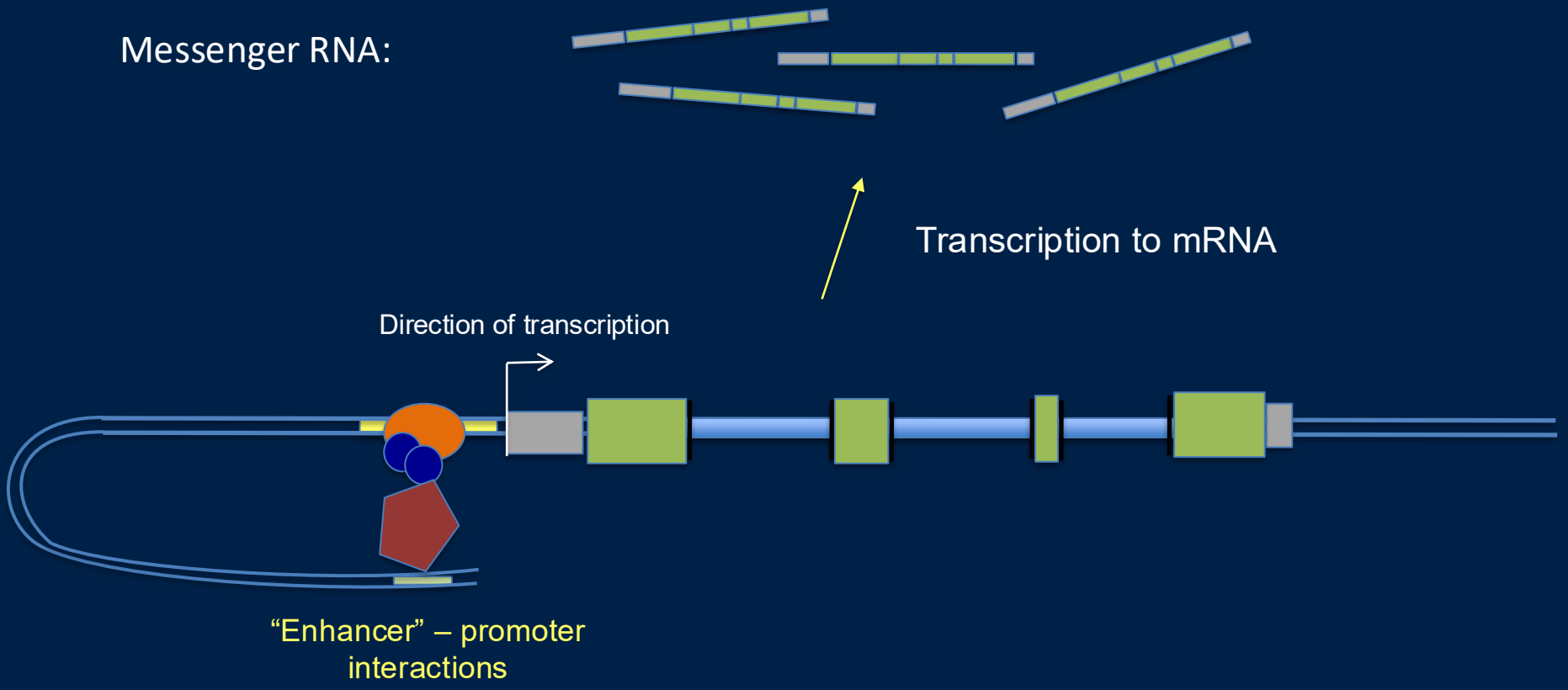
Direction of transcription

"Transcription factors"
must be able to bind.

Cartoon of a gene

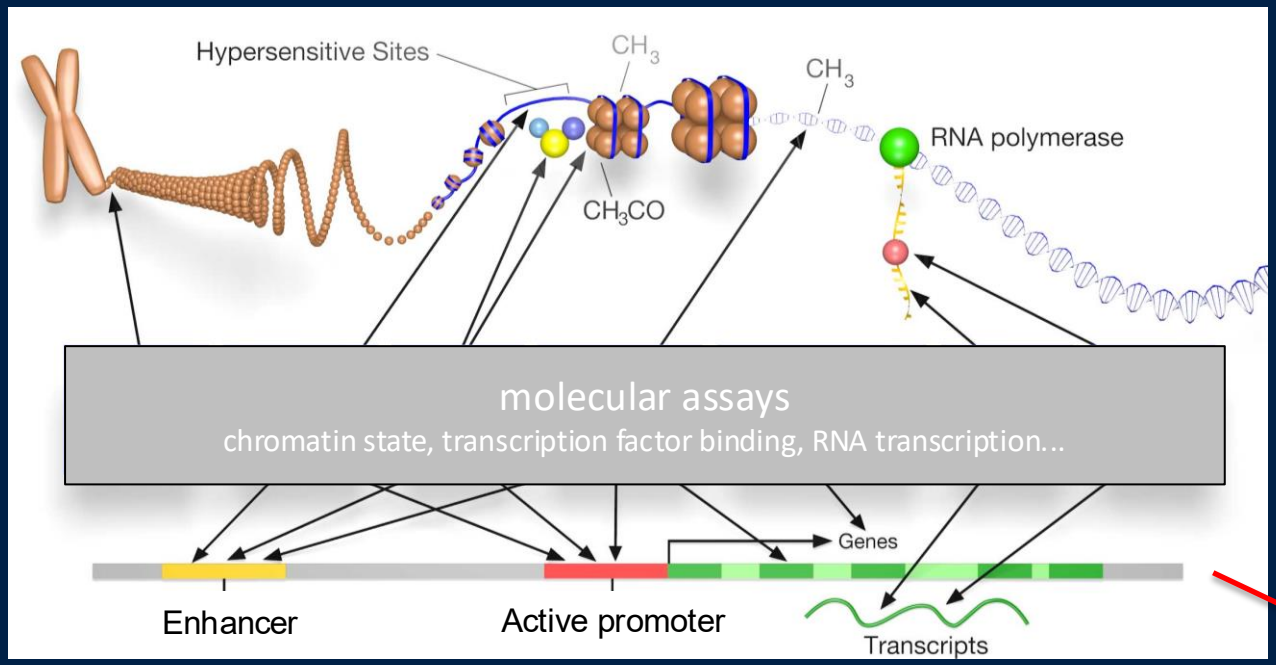


Cartoon of a gene



All this can be measured

Two ways to look at transcription

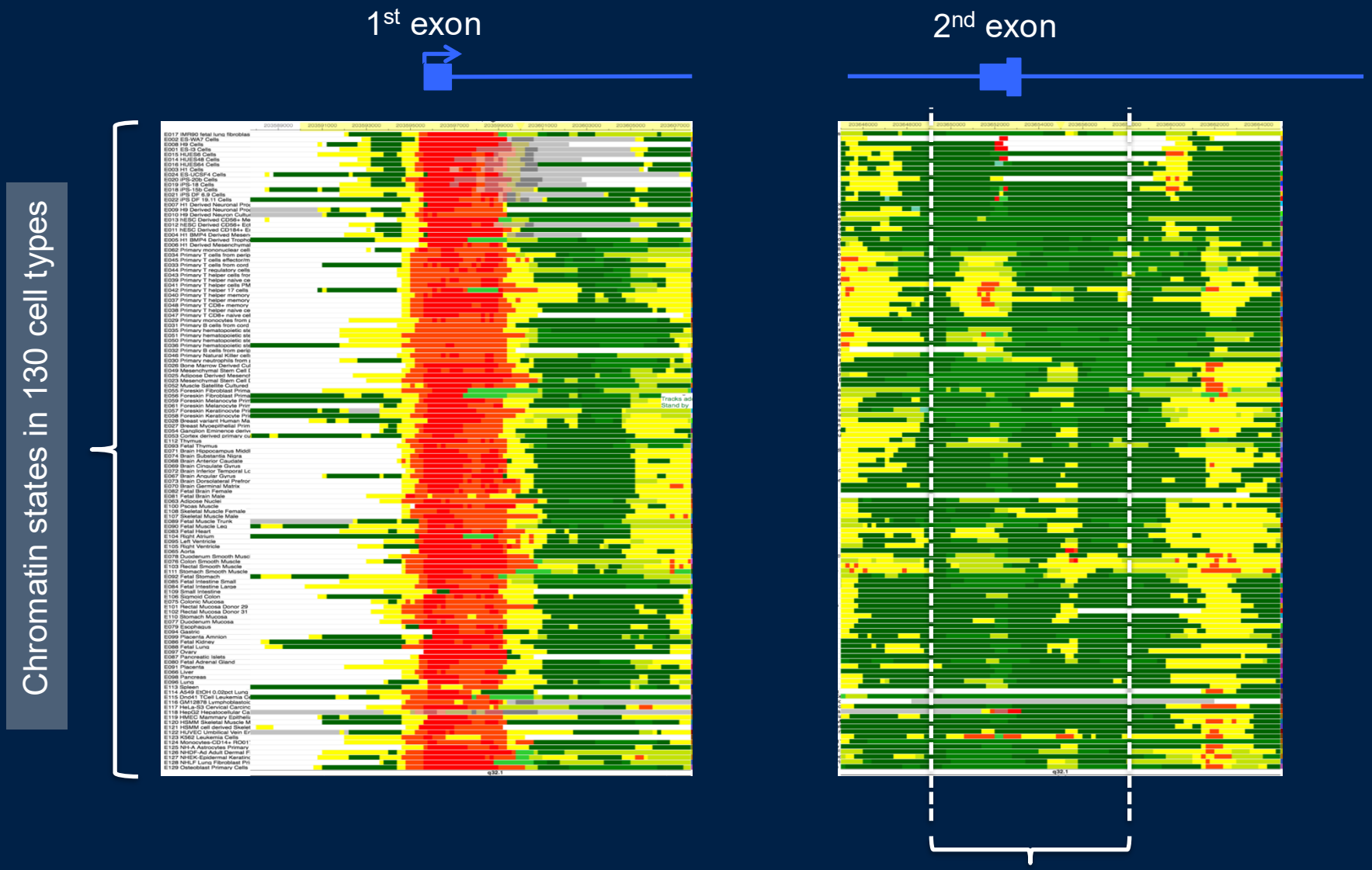


https://www.encodeproject.org

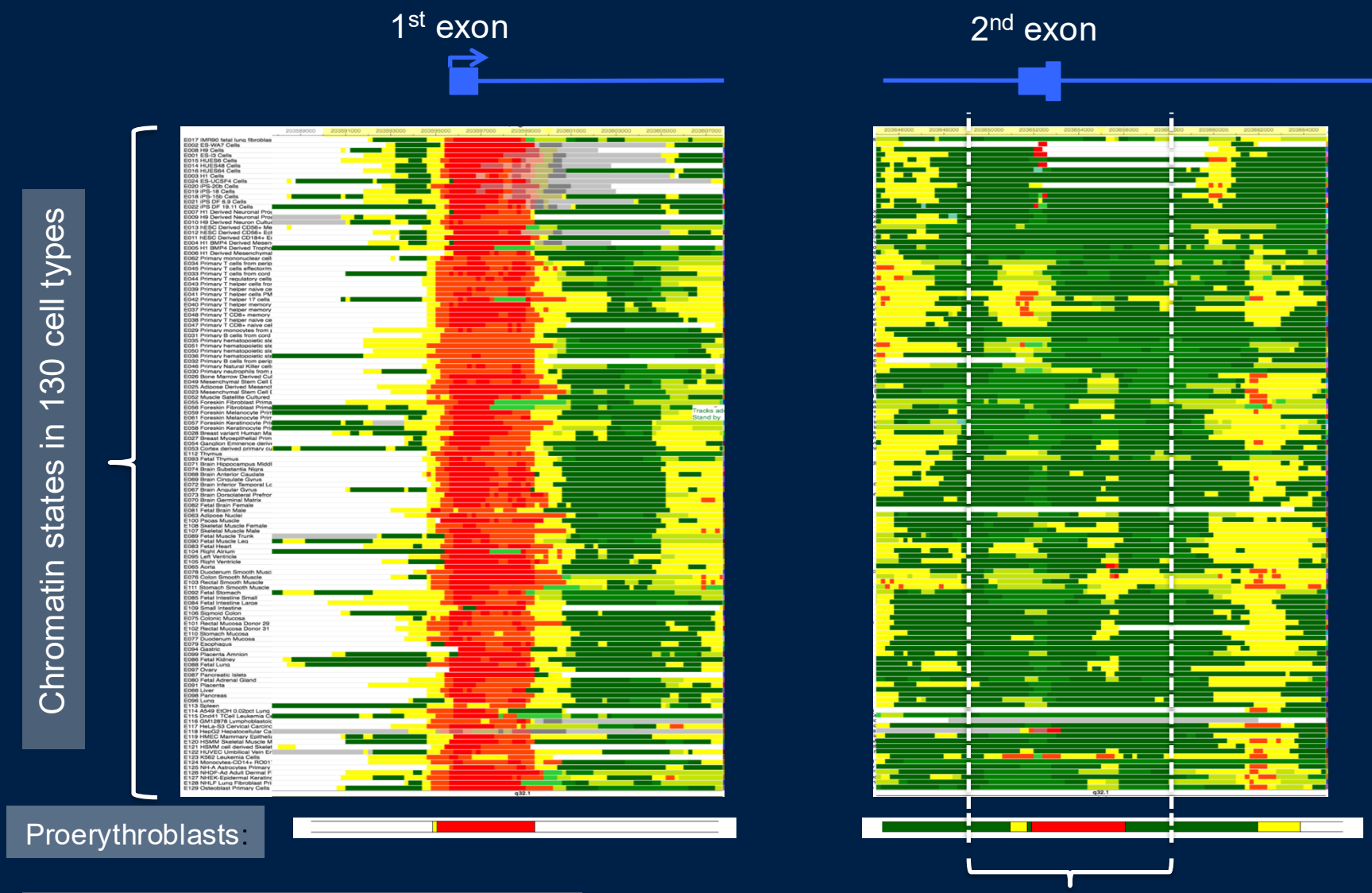
Can look at chromatin state

RNA expression

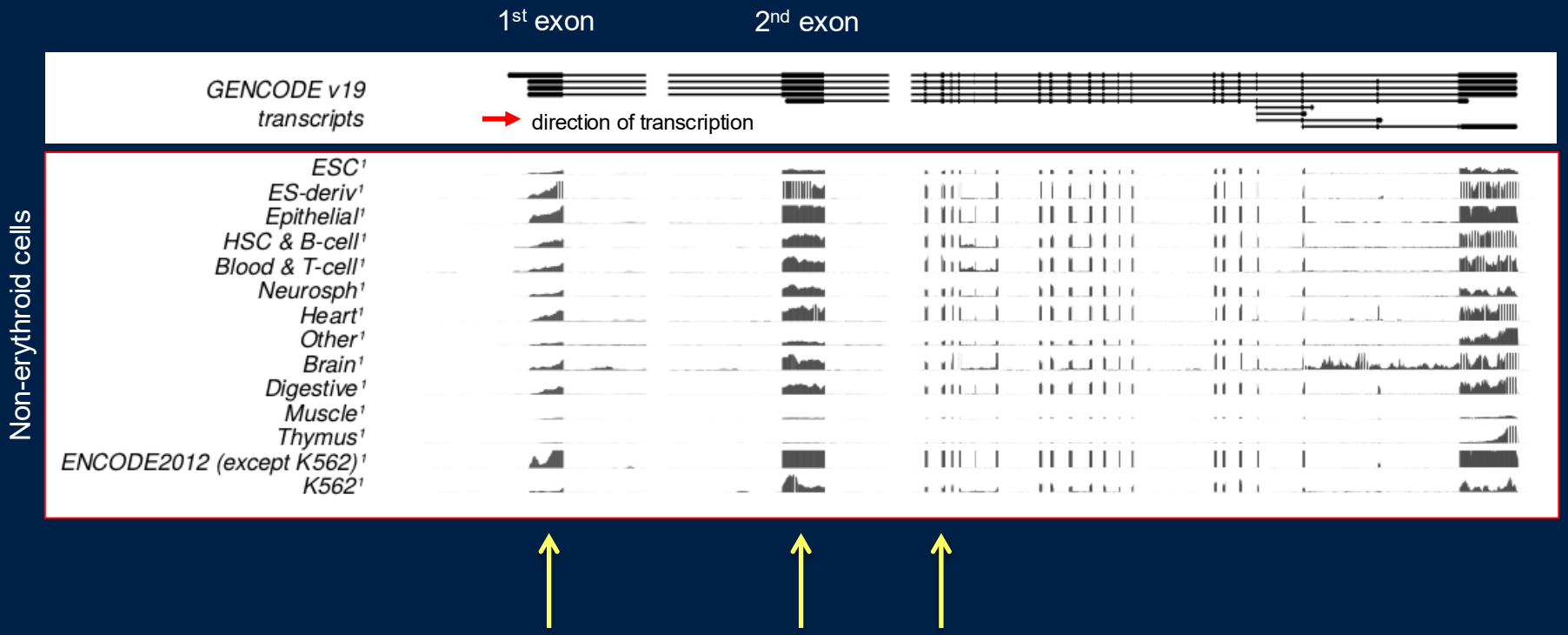
Chromatin state across cell types…



…looks different, maybe, in RBCs?



Expression data (RNA-seq) across cell types

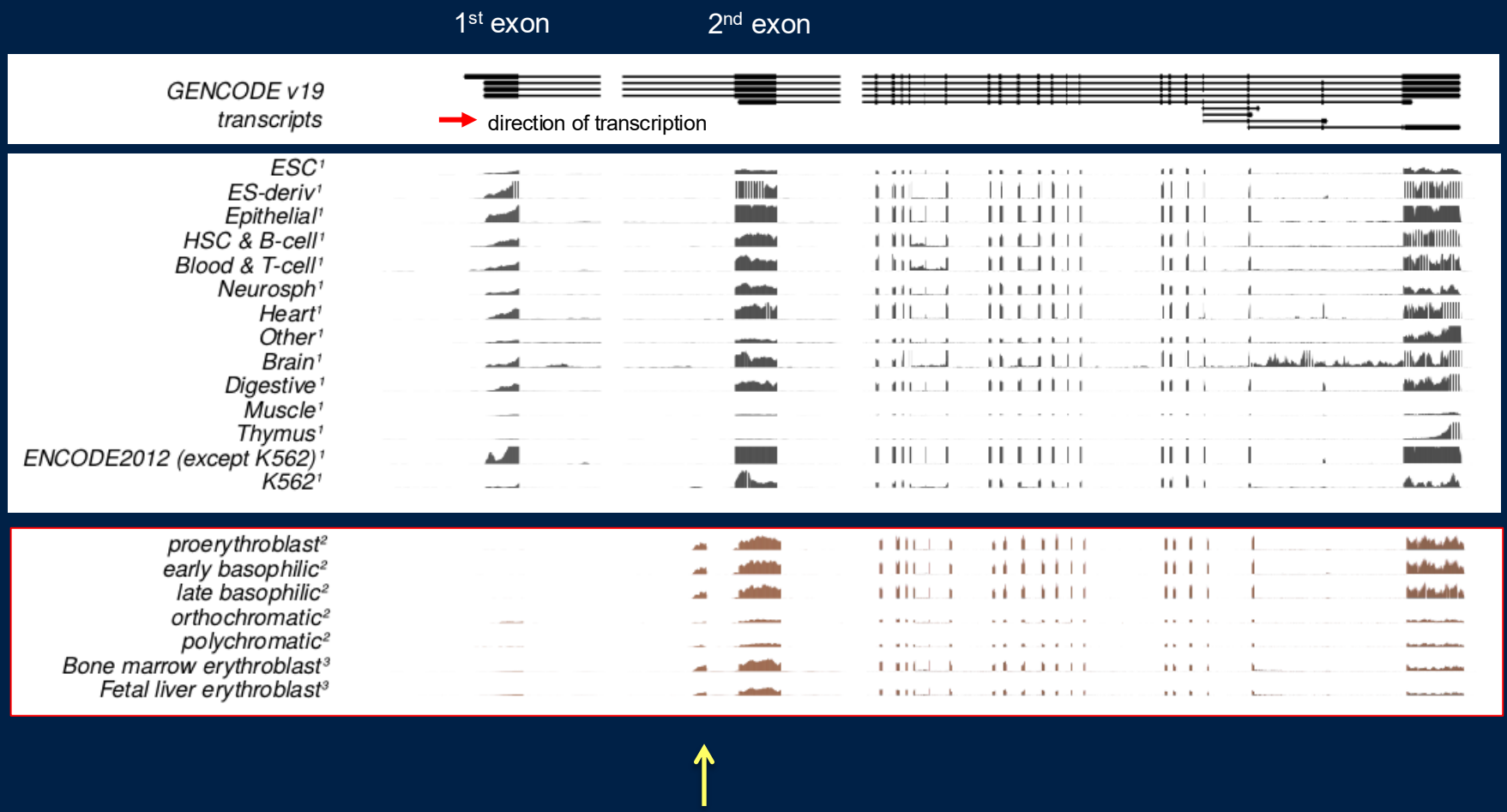


ATP2B4 is expressed essentially in **all cells in the body**

It looks like the gene model says it should

What if we look in red cells?

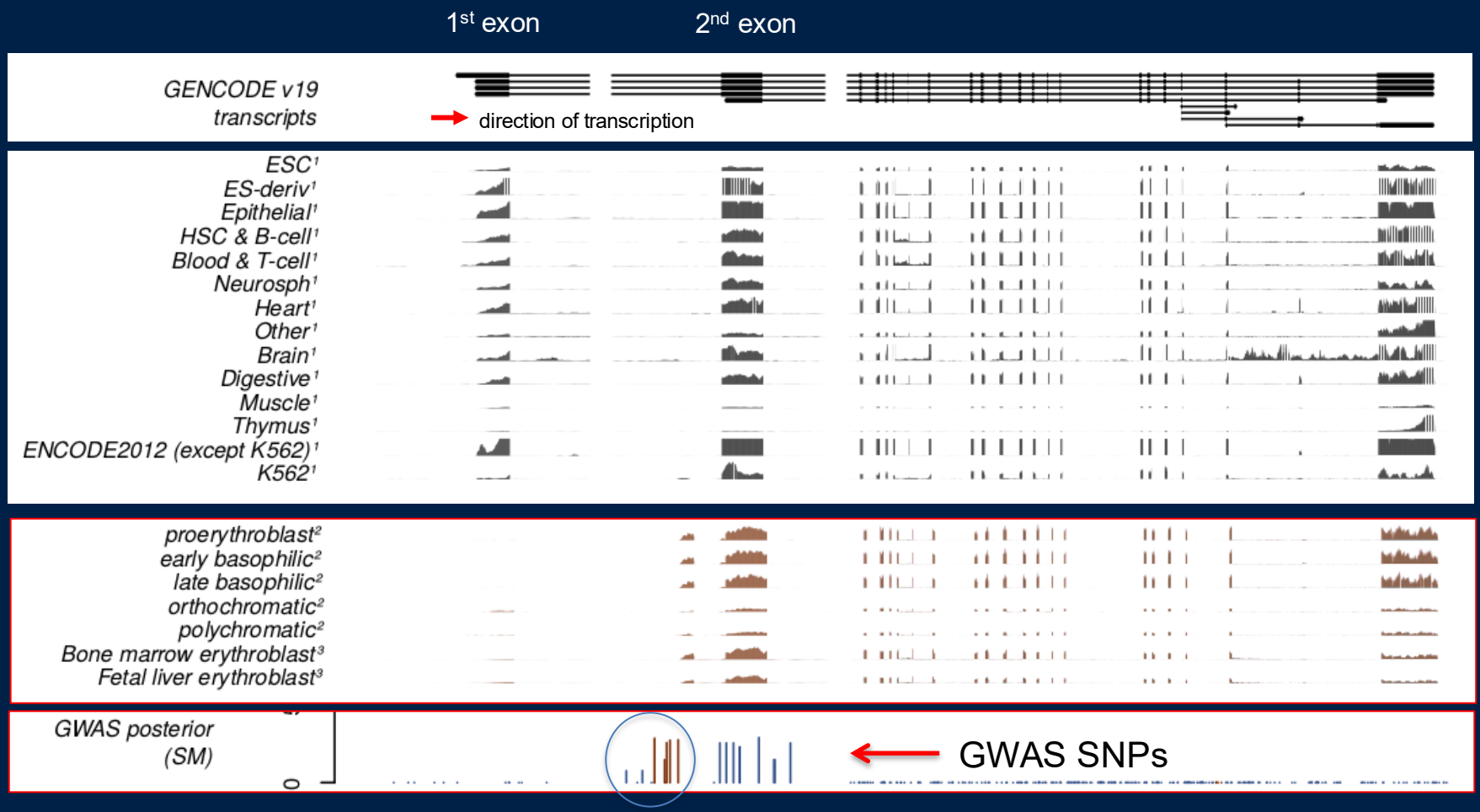
Expression data (RNA-seq) across cell types



Red cell (precursors) show a different pattern

There's a new 1st exon!

Expression data (RNA-seq) across cell types



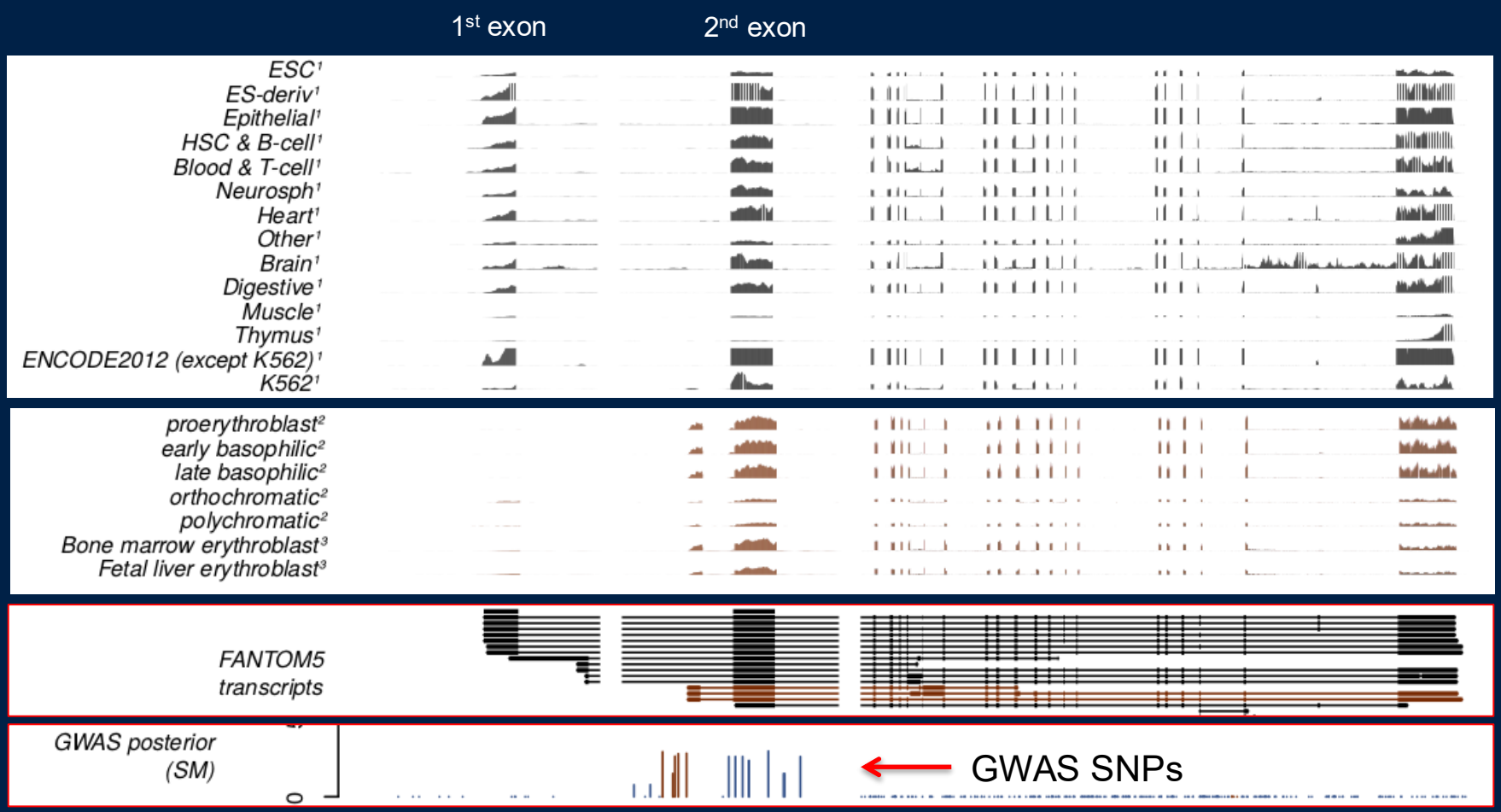
Red cell (precursors) show a different pattern

There's a new 1st exon!

Could these upstream GWAS SNPs be affecting transcription in red cells?

ATP2B4 has an erythroid-specific transcript

Measured RNA transcription (RNA-seq)



Putting together data from a variety of sources suggests the existence of an *alternative transcription start site* near the GWAS signal, but only active in erythrocytes. How can this be?

What is different about RBCs?

Transcription factor (TF) binding helps promote transcription

A major TF in red cells is **GATA1.**

"GATA1 regulates the expression (i.e. formation of the genes' products) of an ensemble of genes that mediate the development of red blood cells and platelets."

- Wikipedia

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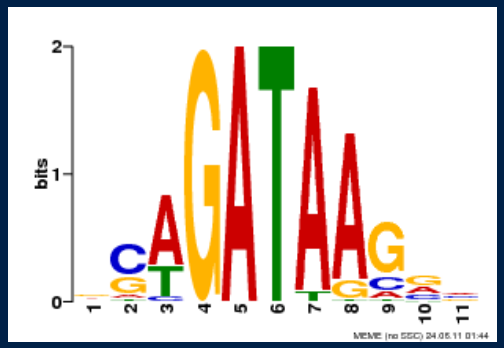


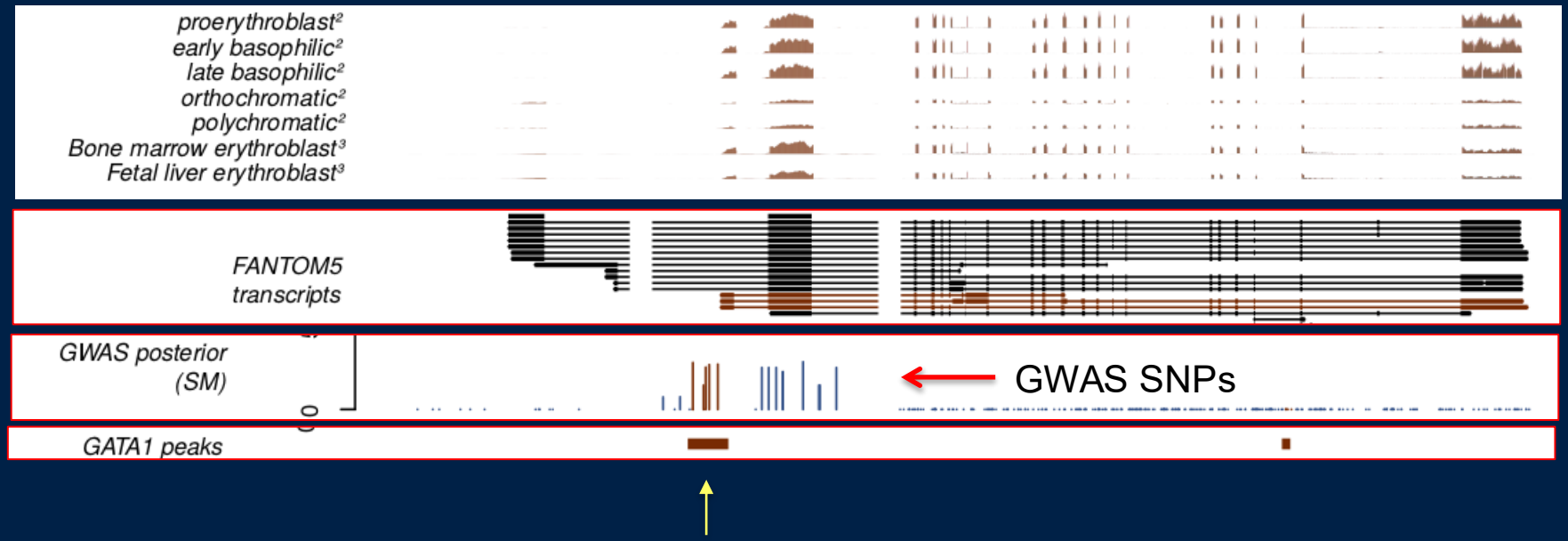
v1.factorbook.org

…so-called because of the **DNA motif** it binds to.

"GATA1 regulates the expression (i.e. formation of the genes' products) of an ensemble of genes that mediate the development of red blood cells and platelets."

- Wikipedia

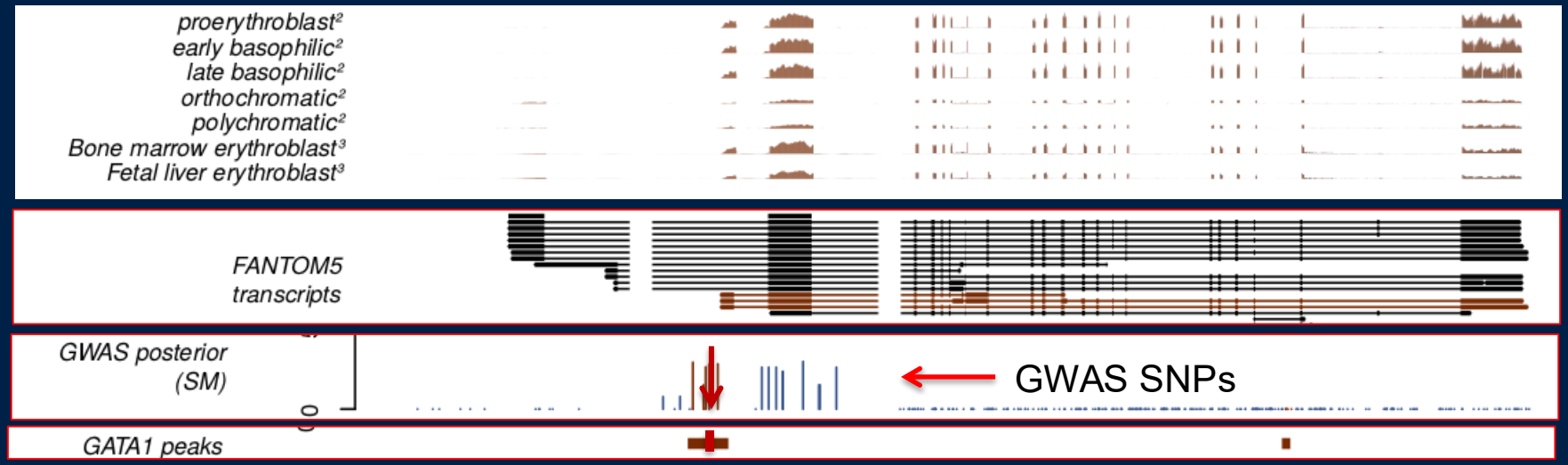
Putting the story together



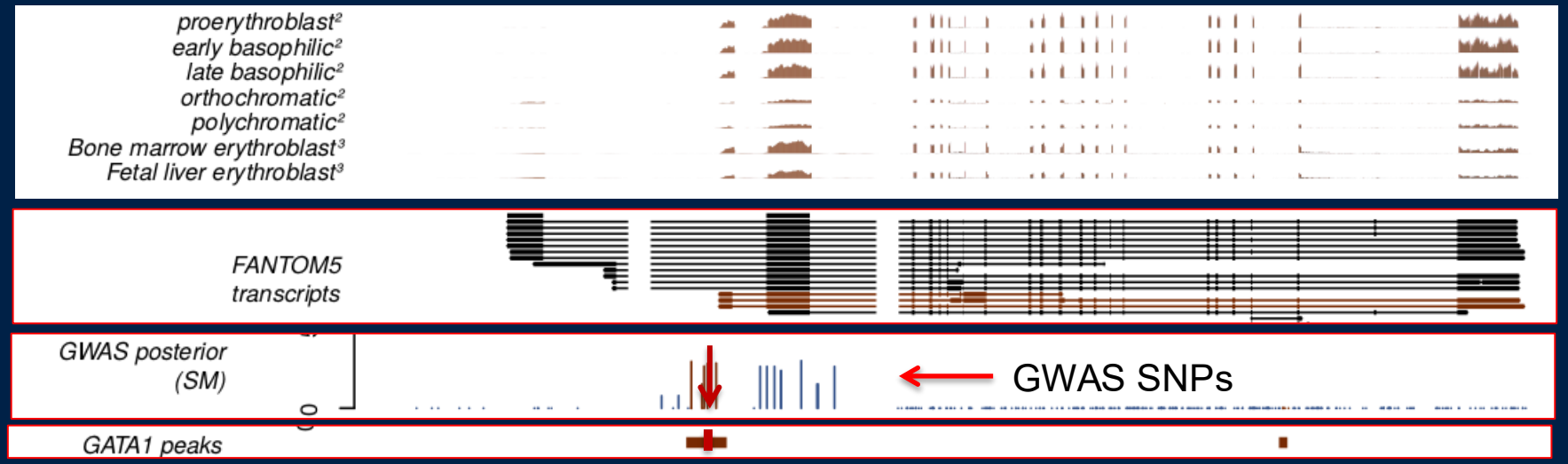
GATA1 does bind this region in erythroid cells

measured using ChIP-seq

Putting the story together



Putting the story together



rs10715451

(malaria-protective allele) ...GGAGCGGTAAGATA... → Predict lower expression

(malaria risk allele) ...GGAGCGATAAGATA... → Predict higher expression

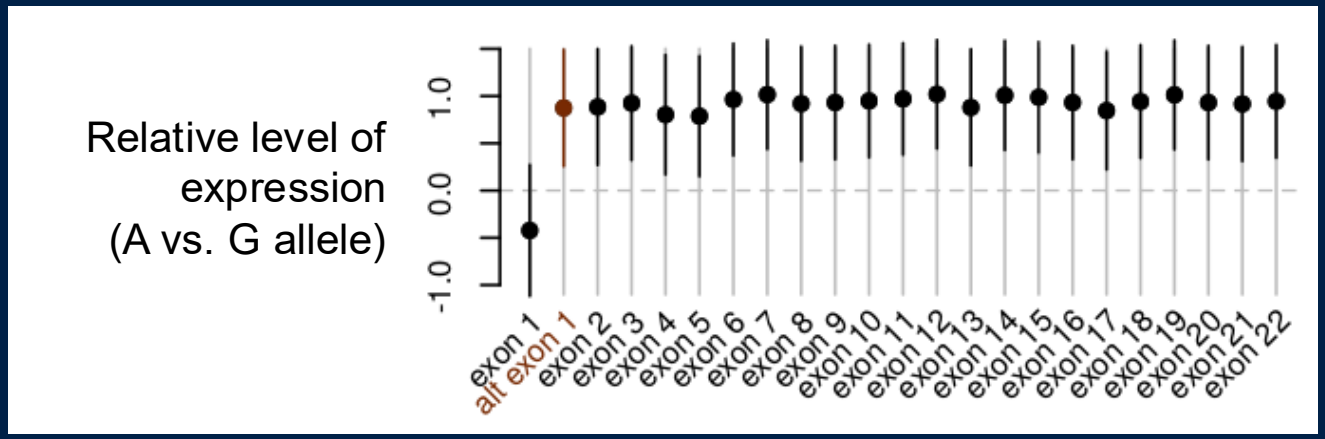
Prediction from all this detective work:

The malaria risk allele causes higher expression of ATP2B4 – and more calcium pumping – in red cells

Probably without affecting other cells in the body

Genetic prediction

This prediction is borne out by RNA-seq data in cells from people with different genotypes.



N = 24

Only limited data is available

This example illustrates the complexities of fine-mapping

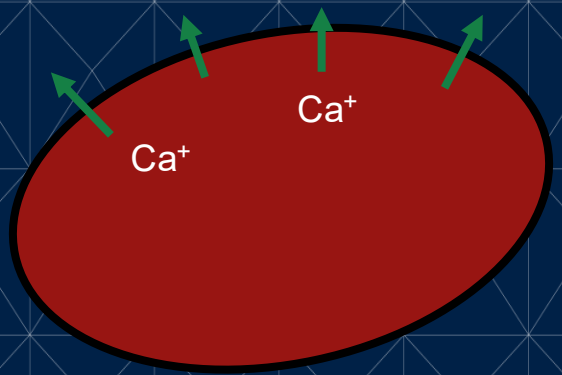
We've gone from a GWAS signal and (putatively) narrowed it down to a possible causal effect of a single SNP, in a single relevant cell type.

To get there we used a raft of functional genomic data – RNA-seq, ChIP-seq, chromatin state models – across different cell types.

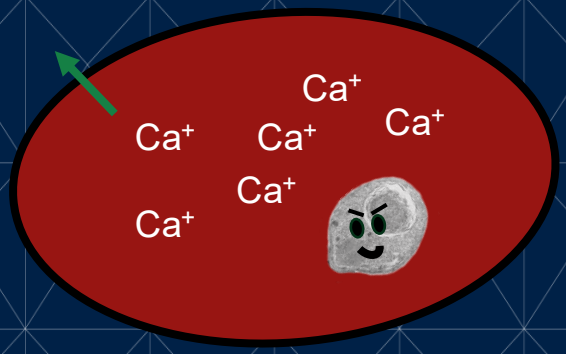
There is one remaining conundrum…

ATP2B4 encodes a calcium pump. It removes Ca^{+} from the cell.

Parasites need calcium to grow. They *should* like the cell on the right…



rs10715451 G allele
associated with **malaria risk**

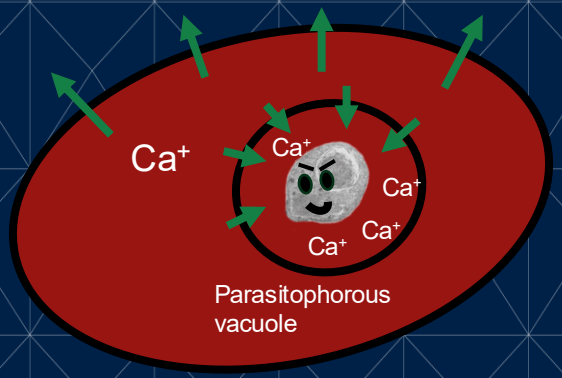


rs10715451 A allele
associated with **malaria protection**

There is one remaining conundrum…

ATP2B4 encodes a calcium pump. It removes Ca^+ from the cell.

Hypothesis: pump gets inverted in the membrane of the parasitophorous vacuole.



rs10715451 G allele
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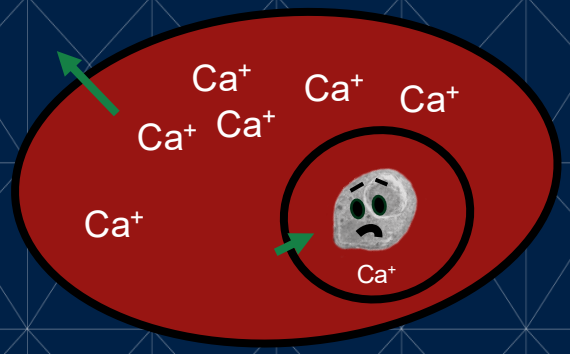
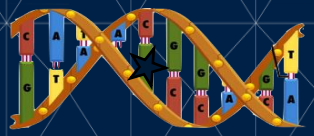


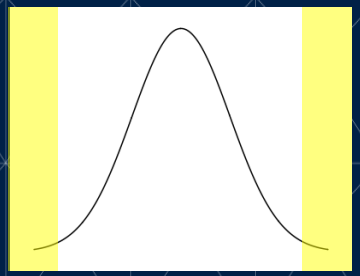
rs10715451 A allele
associated with **malaria protection**

Lessard et al 2017, Zambo et al 2017, Joof 2024

The circle of genetic causation



...passing on DNA, with **mutations** and **recombination**, to new generations...



...whose success is affected by the traits they have...

microarrays, genome sequencing

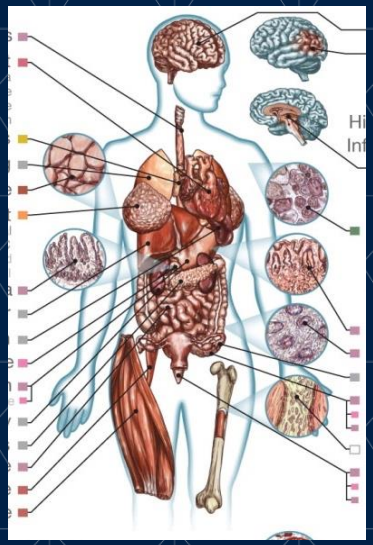
Clinical phenotype measurements

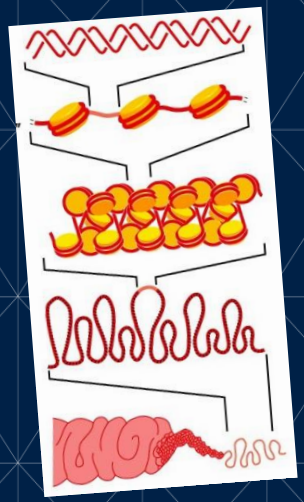
...that gets physically packaged up into chromosomes...

There is complex biology at all stages

And we can measure it.

Biomarker measurements

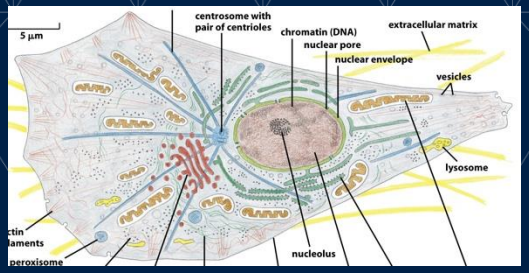




Chromatin state marker assays, ChIP-seq, ...

RNA-seq, spectroscopy, antibody binding

...that combine to make individuals...



...inside cells, where it is **transcribed** to form proteins and other molecules...

...that affect how the cells behave, forming different organs...

Learning biology from GWAS - summary

Non-coding variants

Long-distance interactions in the genome

Changes to gene expression

Polygenic effects (lots of variants involved)

Cell-type / tissue heterogeneity

Pleiotropy (a variant affects lots of phenotypes at once)

Genetic interactions

Host-pathogen interactions

Repetitive DNA / repeat expansions

Genome structural variation

Genome evolution

Anything that can happen, it does happen.

Prospective cohort studies

A new crop of studies aims to create a database of deep genotype, phenotype, and exposure data across large cohorts of individuals sampled from the population or from health services. Examples:

Precision Medicine Initiative, All of Us, Million Veteran's Programme (US)

CartaGene (Canada)

China Kadoorie Biobank

FinnGen (Finland)

UK Biobank

The 100,000 genomes project (UK)

+ Our Future Health

In partnership with NHS

[redacted]

1,885,350

people are already taking part in the UK's largest health research programme.

(As of 26 September 2024)

To all residents,

An opportunity to take part in research and learn new information about your blood pressure and future risk of disease.

You are invited to take part in Our Future Health, the UK's largest ever health research programme. If you take part, you will have the chance to find out more about your health now, and your risk of developing some diseases in the future.

Today, too many people spend many years of their life in poor health. Our Future Health aims to help prevent, detect and treat diseases earlier. Diseases like dementia, cancer, diabetes, heart disease and stroke.

Our Future Health needs up to five million people. Everyone aged 18 and over living in the UK is eligible to take part.

Taking part includes answering some online questions about yourself, providing a blood sample, and having your blood pressure measured at a local clinic.

In the future you will have the option to receive information on your risk of some diseases including diabetes, heart disease and some cancers. This will be calculated using the information you provide and analysis of the DNA in your blood sample.

Scan this QR code for more info and to sign up

Or visit **ourfuturehealth.org.uk/join/0518**

£10 voucher

Sign up using the QR code or website link above and you will be eligible for a £10 voucher to recognise the time and effort of volunteering. You can find more information on the back of this letter.

You can also share this invitation with other members of your household.
If you have any questions, please call 0808 501 5634 or email support@ourfuturehealth.org.uk

Yours sincerely,

Raghib Ali OBE MD FRCP(UK)
Chief Medical Officer, Our Future Health
NHS Consultant in Acute Medicine

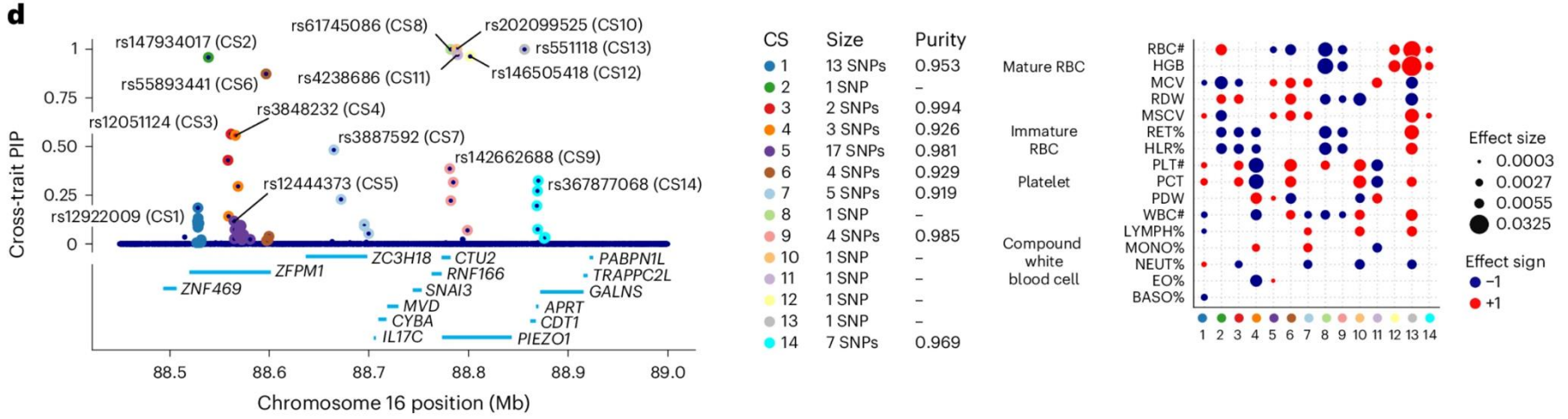
Professor Sir John Bell GBE, FRS
Chairman, Our Future Health

+ Our Future Health

https://ourfuturehealth.org.uk

Pleiotropy example

across blood cell traits



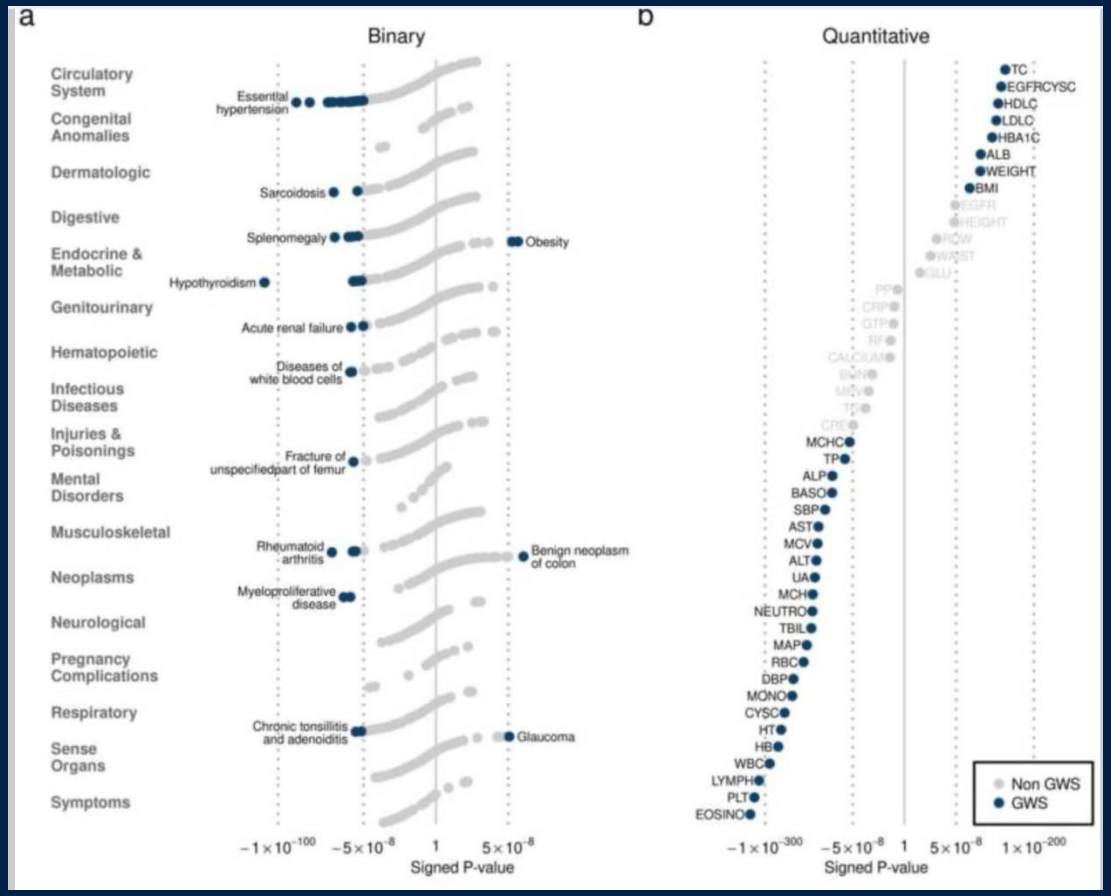
One genome region, 16 blood cell traits

UK Biobank

Zou et al 2026 doi:10.1038/s41588-025-02486-7

Another pleiotropy example – “PheWAS”

across > 1,100 clinical traits



One SNP, > 1,1000 traits

In SH2B3 which along with ABO is one of the most pleiotropic genes.

Levin et al doi: 10.1101/2025.04.18.25326074

Learning objectives

Understand a genome-wide association study (GWAS) and the concept of a hypothesis-free approach to studying genetic associations.

Have a working knowledge of the different steps involved in the conduct of GWAS, including study design, quality control and basic analyses.

Be able to interpret and critically appraise evidence from genome-wide association studies.

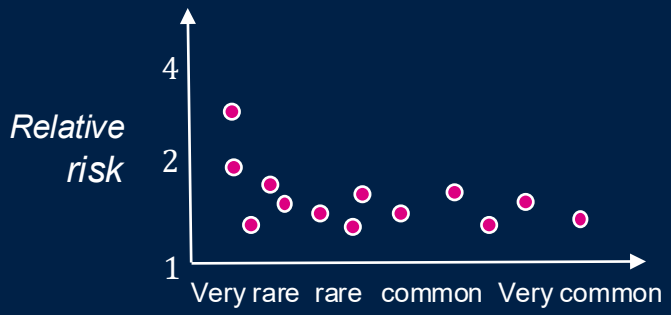
Understand the relevance of replication, meta-analysis and consortia, and multi-ancestry approaches, in genome-wide association studies.

Appreciate the use of post-GWAS analyses including fine mapping, gene and pathway analyses, and the concept of causal variants.

Main points in this lecture

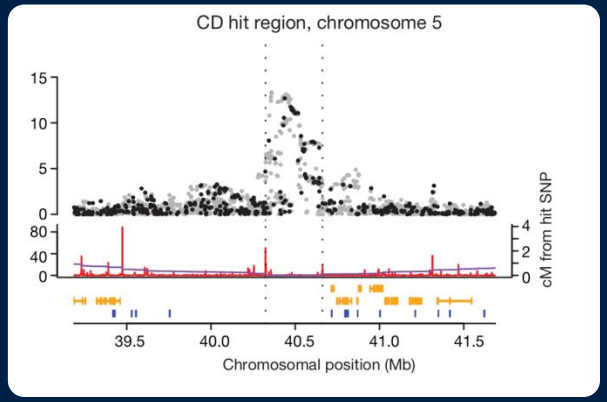
How polygenic do traits get, anyway?

Answer: Very!

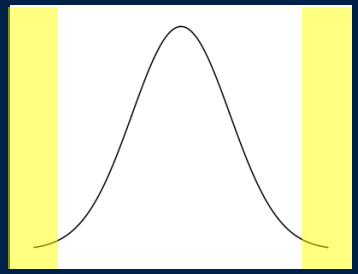


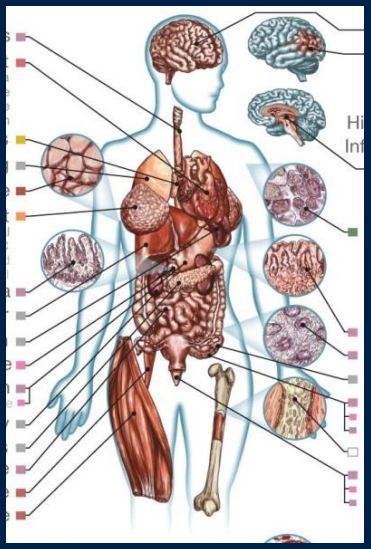
Extracting biological information from GWAS is hard!

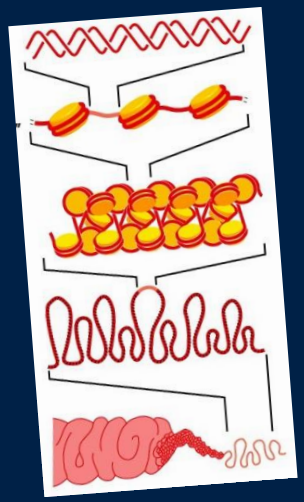
Pathway analysis – fine mapping - pleiotropy



We need highly trained people like you!







Thanks for listening!