

# Epigenetics of Cardiovascular Diseases

A primer in epigenetics of cardiovascular disease

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UMC Utrecht



# What we'll discuss today...

- Recapture Some Basic Genetics - again
  - Human Genome & Genetic Variation
  - GWAS & Statistics
  - Coronary artery disease
- Intermezzo
- Basic concepts epigenetics
- Our research





Recapturing Some Basic Genetics

# THE HUMAN GENOME



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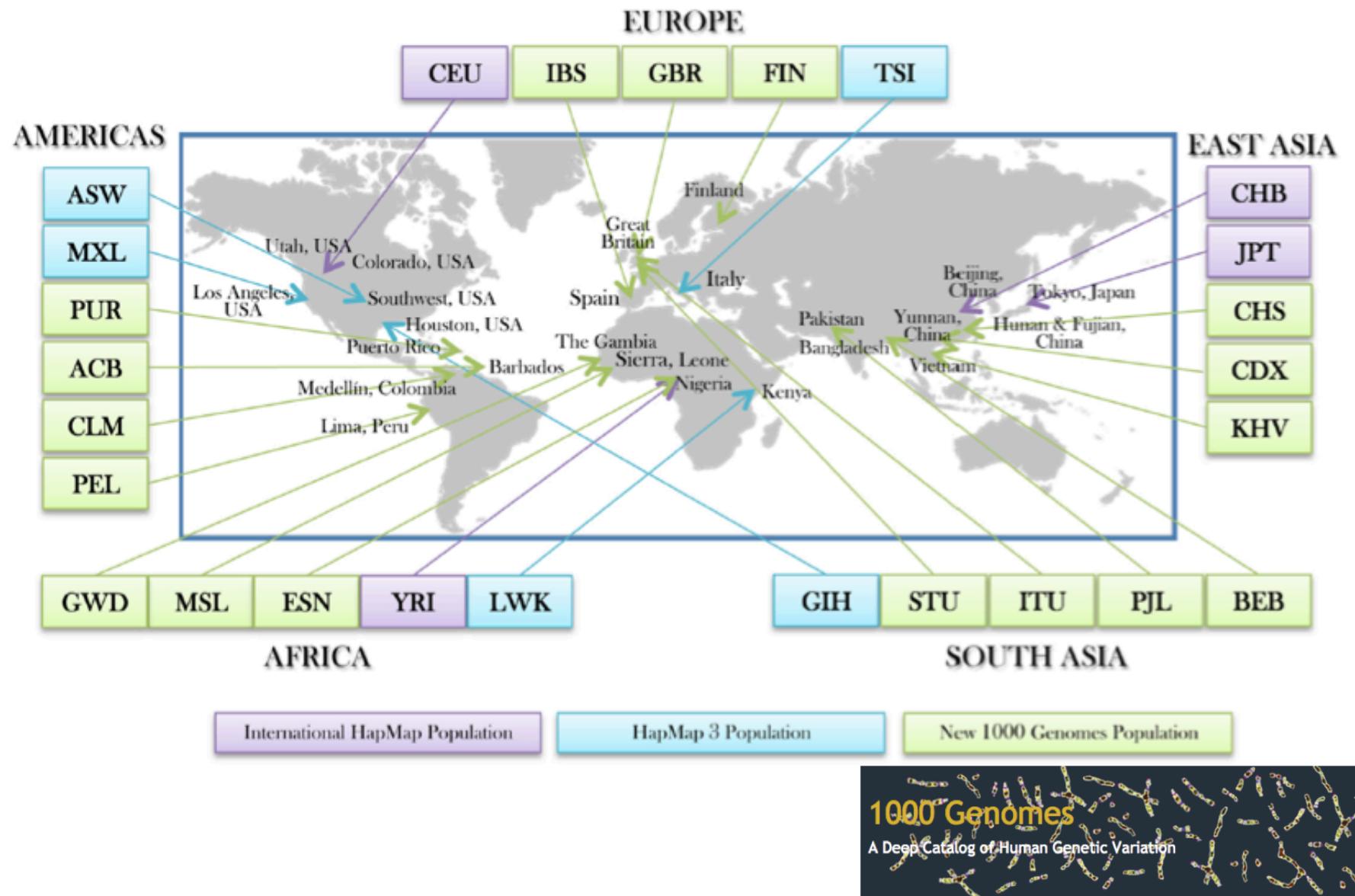
# We are all equally unique

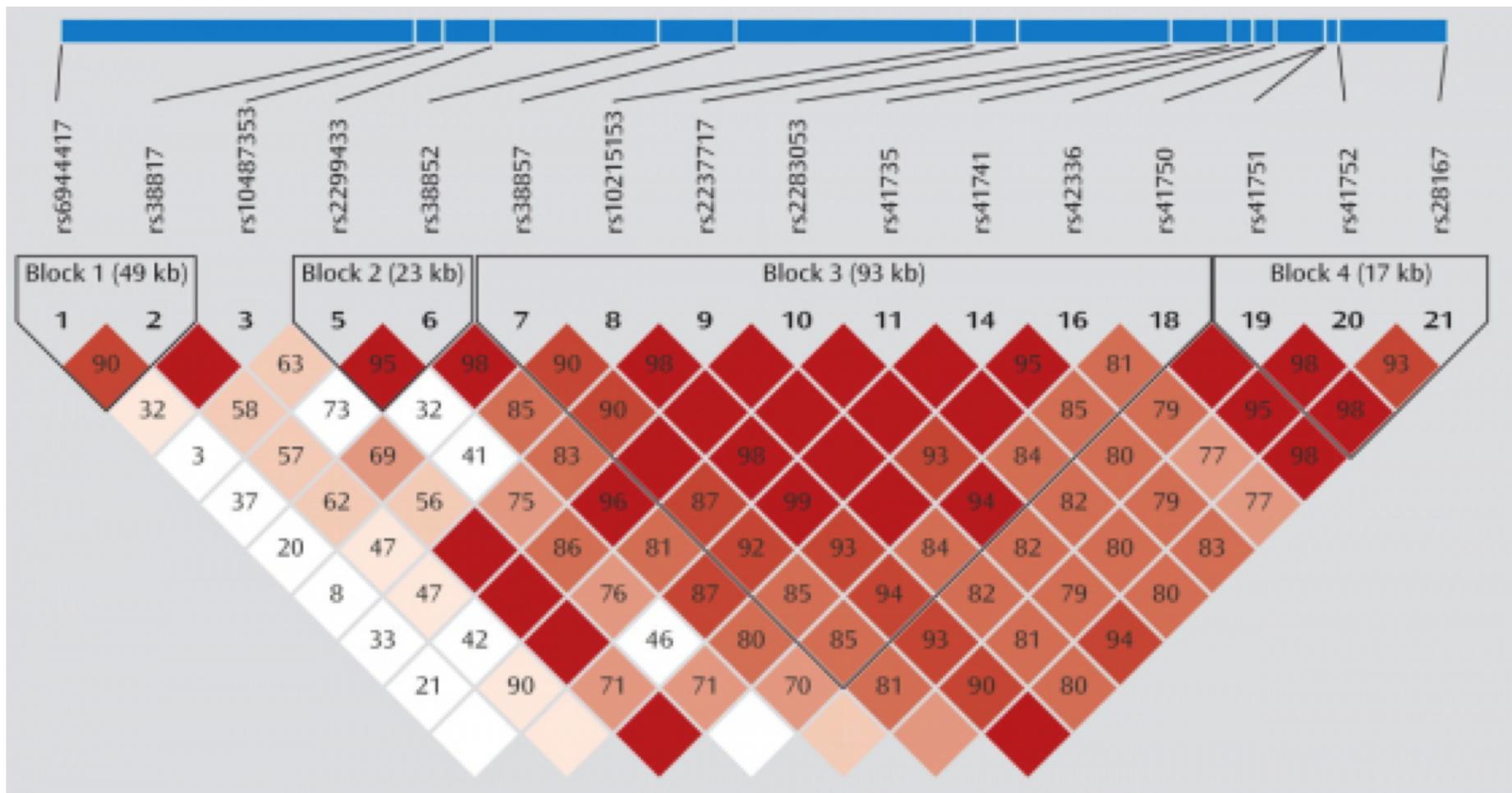
**~100 million genetic variations are known to date**

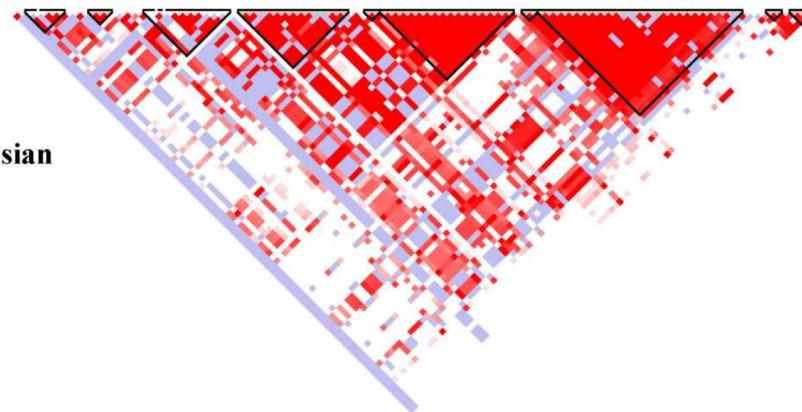
**among ~3 billion base pairs in the human genome**



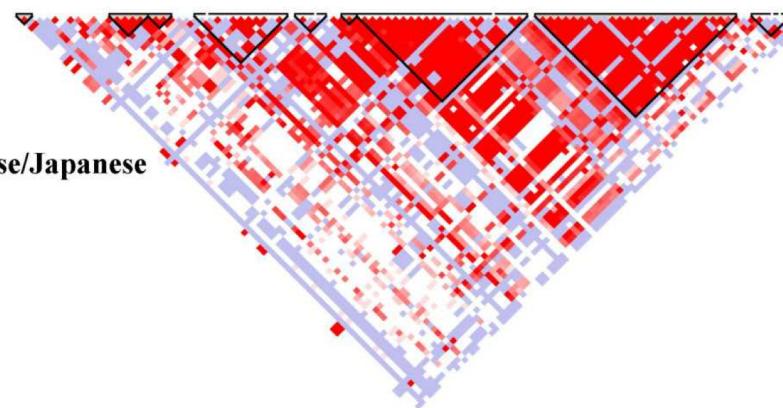
# The 1000 Genomes Project



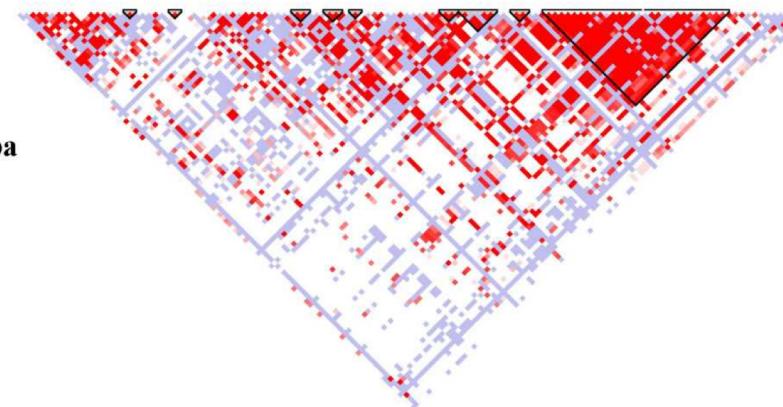




Caucasian



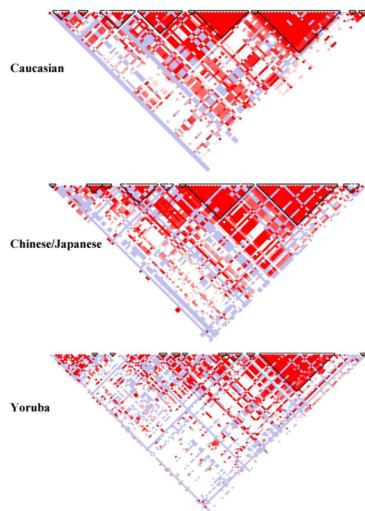
Chinese/Japanese



Yoruba



# These genetic variants, i.e. single-nucleotide polymorphisms (SNPs), are correlated and form *haplotypes*



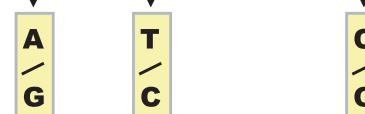
a SNPs

SNP  
↓  
Chromosome 1 AACAC**C**GCCA.... TTCC**G**GGGT**C**.... AGTC**G**ACCG....  
Chromosome 2 AACAC**C**GCCA.... TTCC**G**AGGT**C**.... AGTC**A**ACCG....  
Chromosome 3 AACAC**T**GCCA.... TTCC**G**GGGT**C**.... AGTC**A**ACCG....  
Chromosome 4 AACAC**C**GCCA.... TTCC**G**GGGT**C**.... AGTC**G**ACCG....

b Haplotypes

Haplotype 1 **C**TC**A**AAAG**T**ACGG**T**TCAGG**C**  
Haplotype 2 **T**TG**A**TT**G**CG**C**AAAC**A**GT**A**ATA  
Haplotype 3 **C**CC**G**AT**T**GT**G**ATA**T**CTGG**T**  
Haplotype 4 **T**CG**A**TT**C**CG**G**GG**T**TCAG**A**C

c Tag SNPs



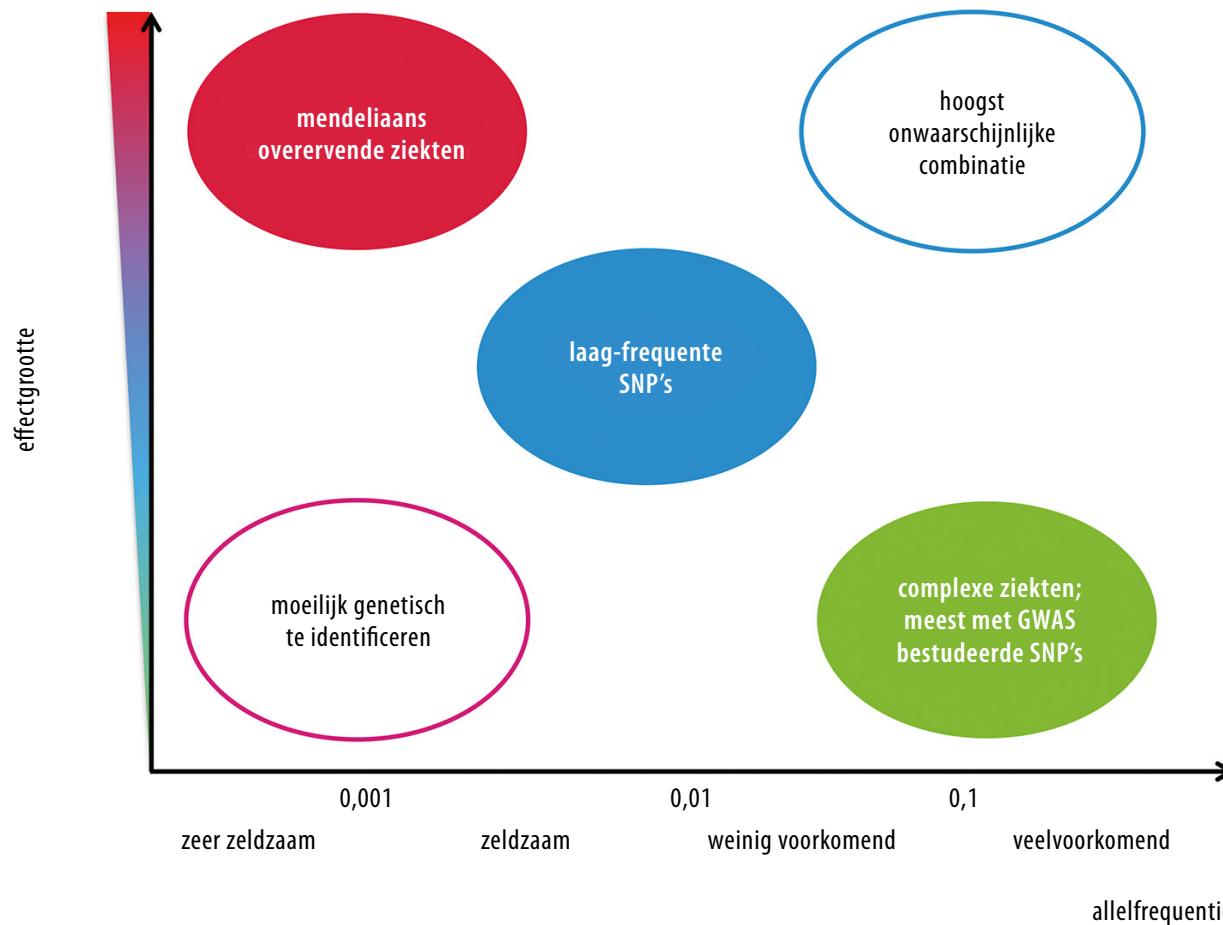
The International HapMap Project. *Nature*; 426:789-796; 2003

Pe'er I. *Genet Epidemiol*; 32(4):381-385; 2008

Dudbridge F. *Genet Epidemiol*; 32(3):227-234; 2008

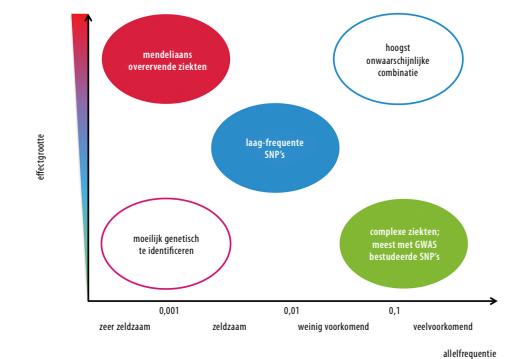
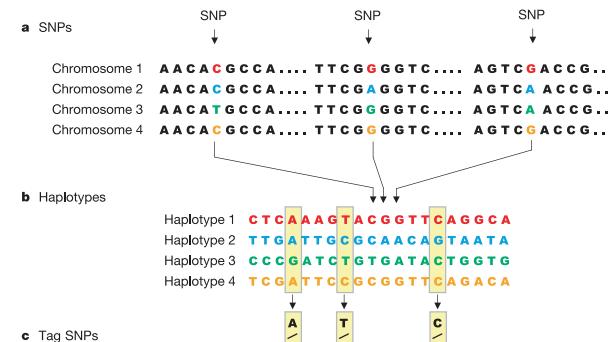


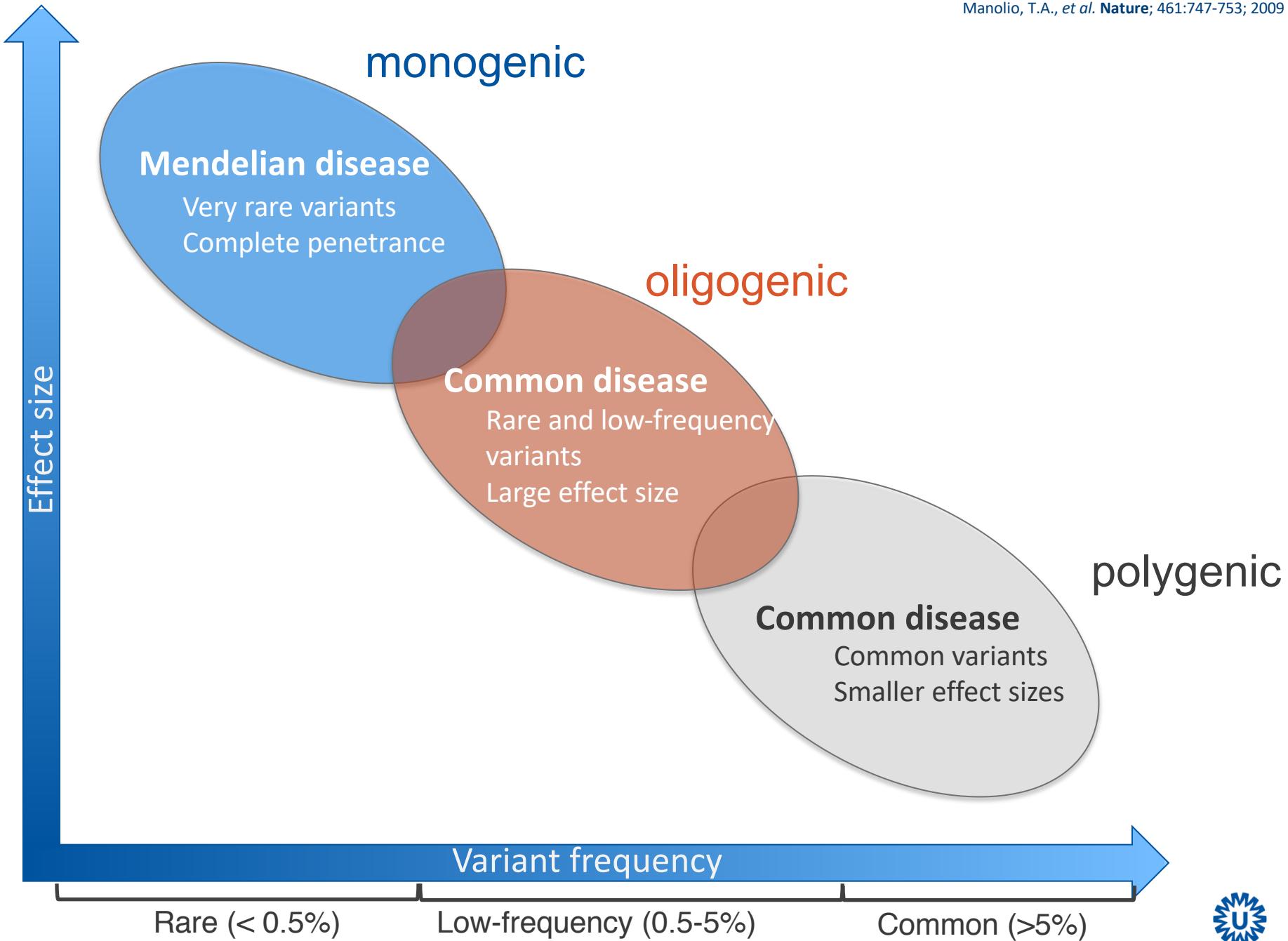
# Common diseases and traits are complex and polygenic by nature many SNPs are involved with small effects



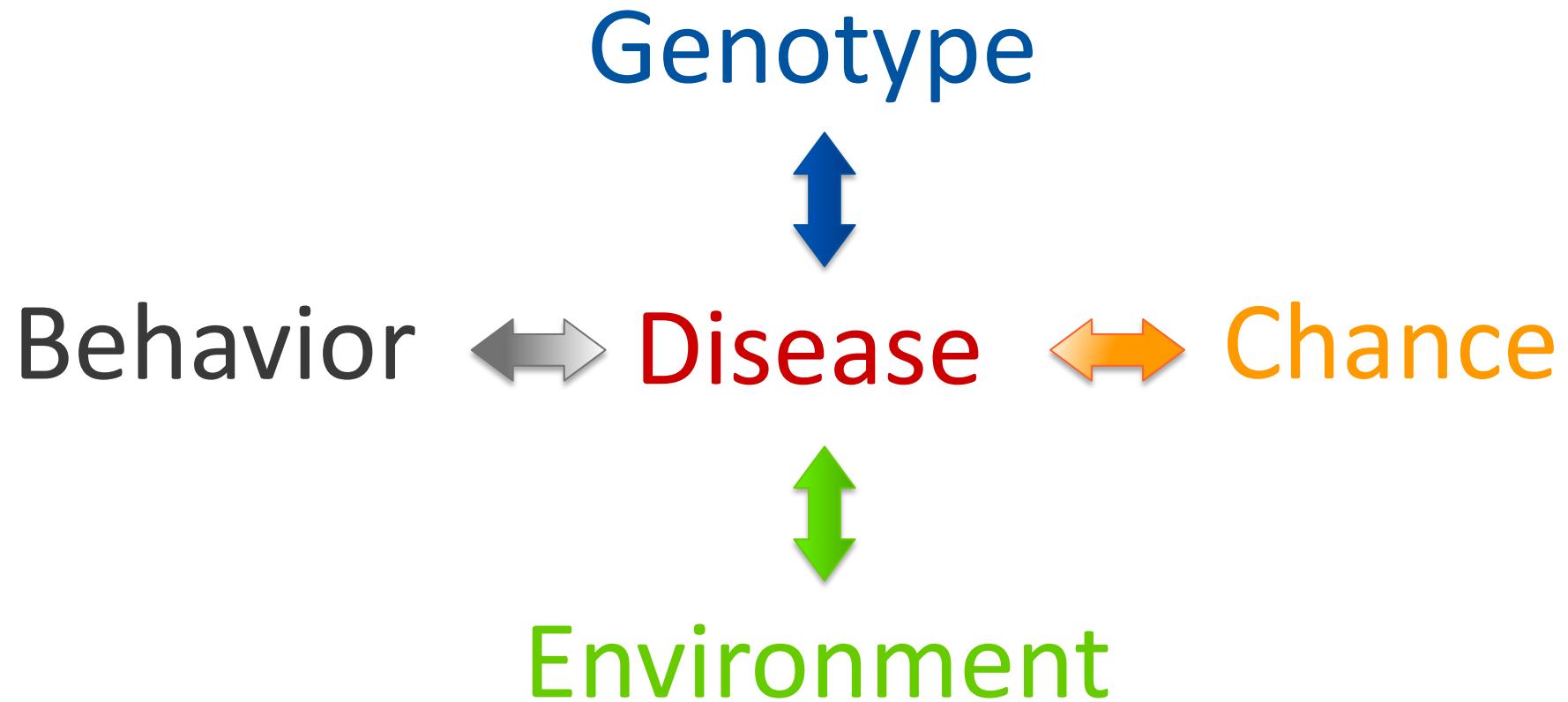
# So now you know 3 things

1. We are all equally unique: ~100 million genetic variations are known to date, among ~3 billion base pairs in the human genome
2. These genetic variants, i.e. single-nucleotide polymorphisms (SNPs), are correlated and form *haplotypes*
3. Common diseases and traits are complex and polygenic by nature: many SNPs are involved with small effects

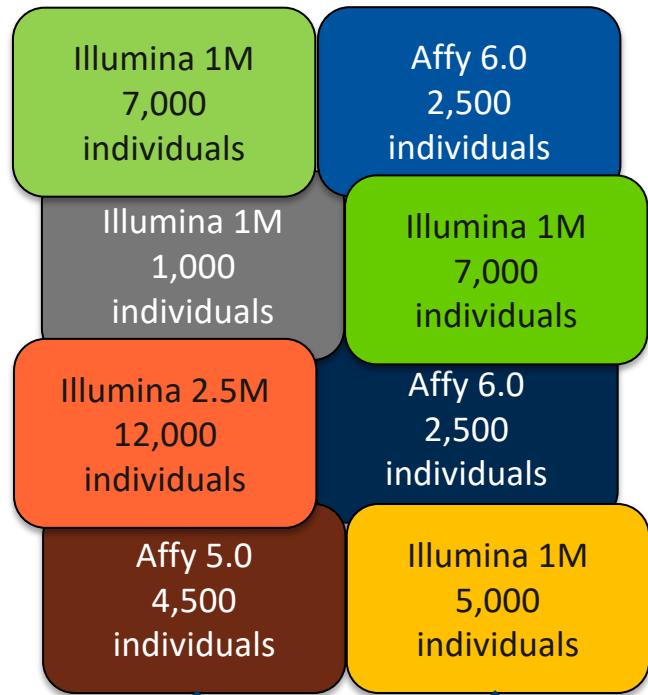




## Many factors influence complex traits and common disease

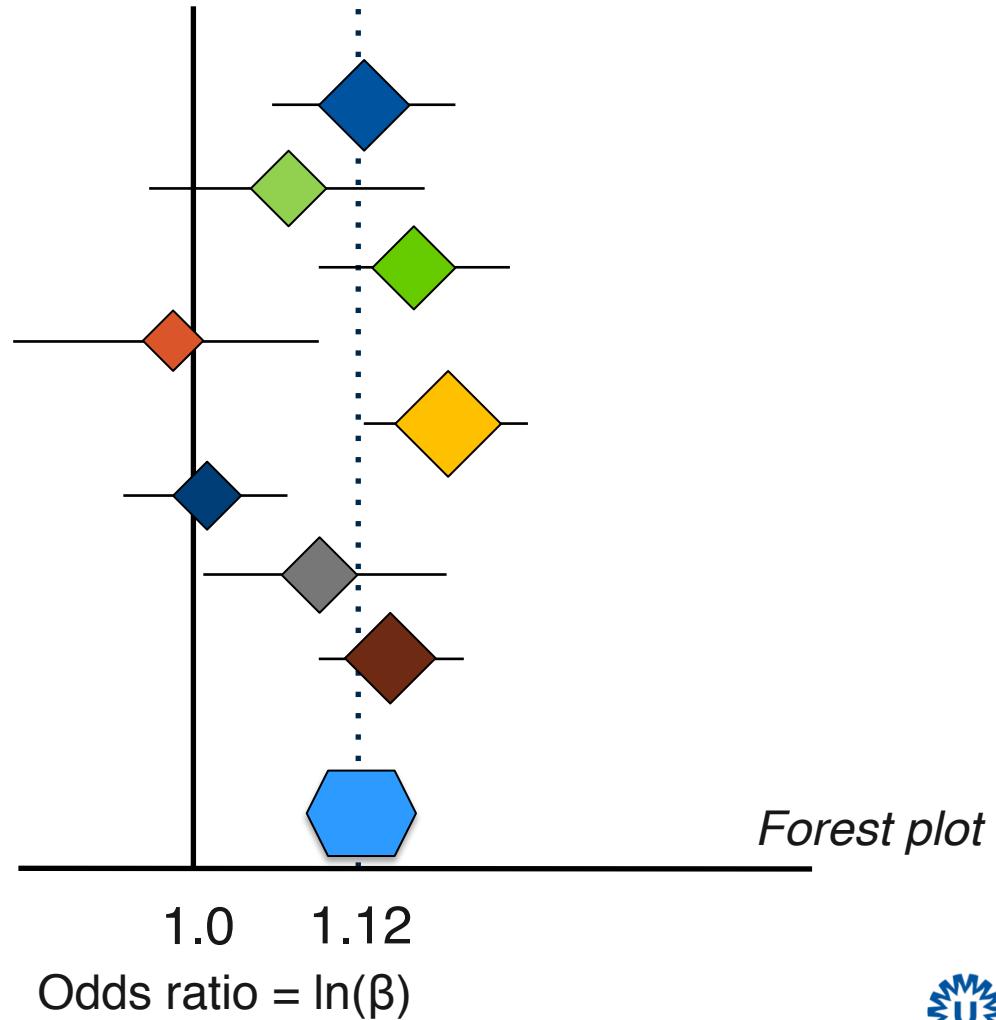


# Combining GWAS datasets



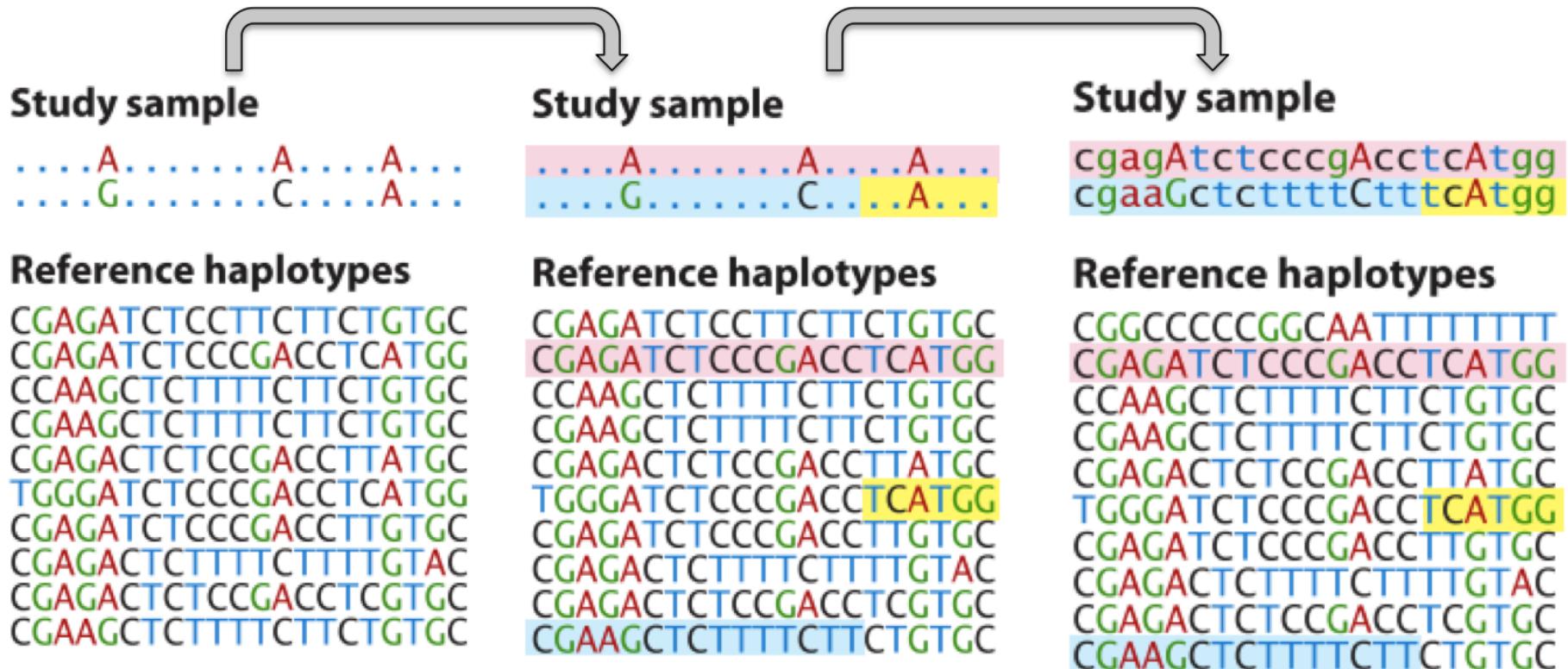
Imputation  
↓  
Meta-analysis of GWAS

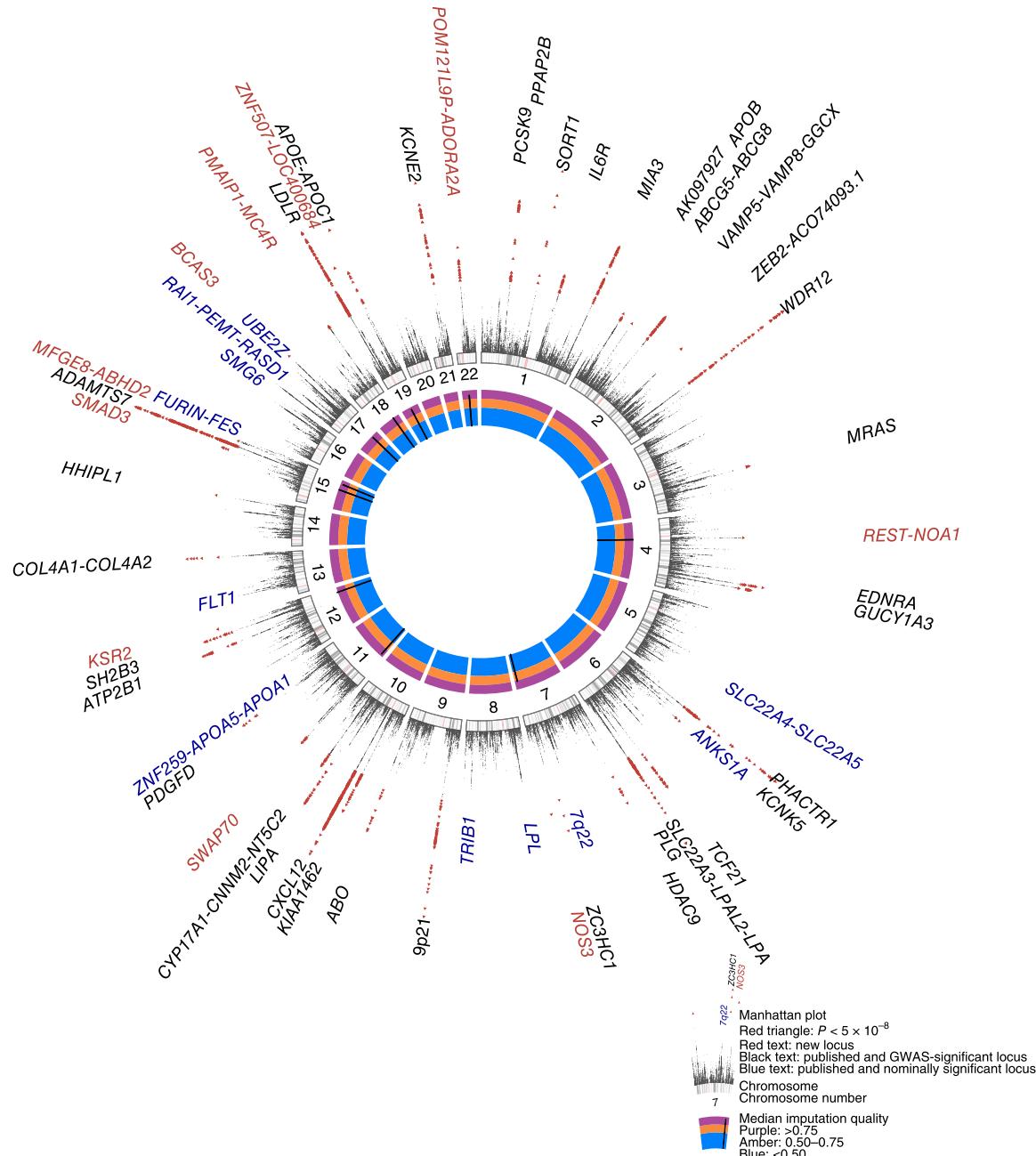
## Results for one SNP



# Imputation

- Imputation: infer untyped genotypes based on a suitable reference panel of well-characterized and validated genotypes





## large patient sample

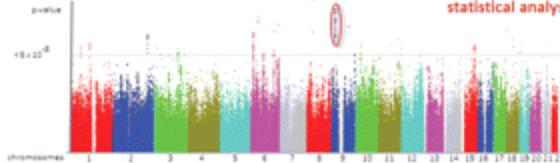


## large control sample



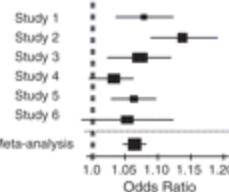
simultaneous genotyping of > 500,000 SNPs

Chr. 1

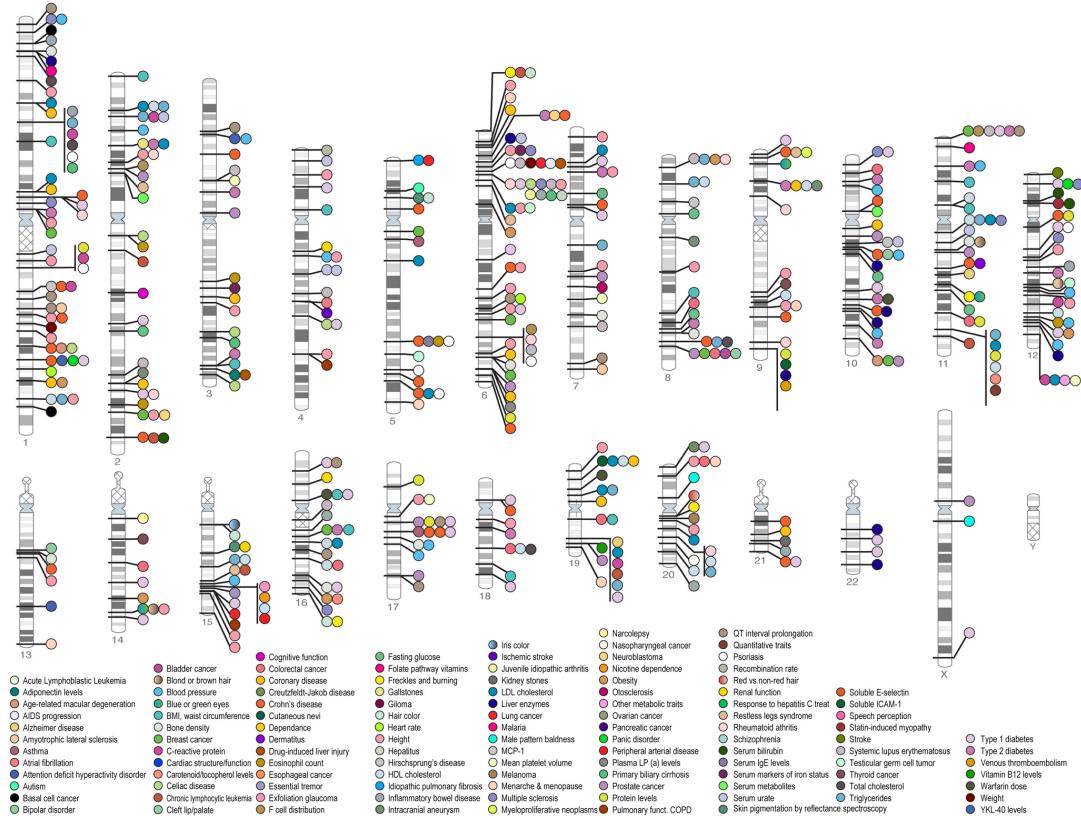


Chr. 22

statistical analysis



Independent replication of top results





# INTERMEZZO



## Let's count...

- Tongue rollers
- Asparagus smelling





# EPIGENETICS



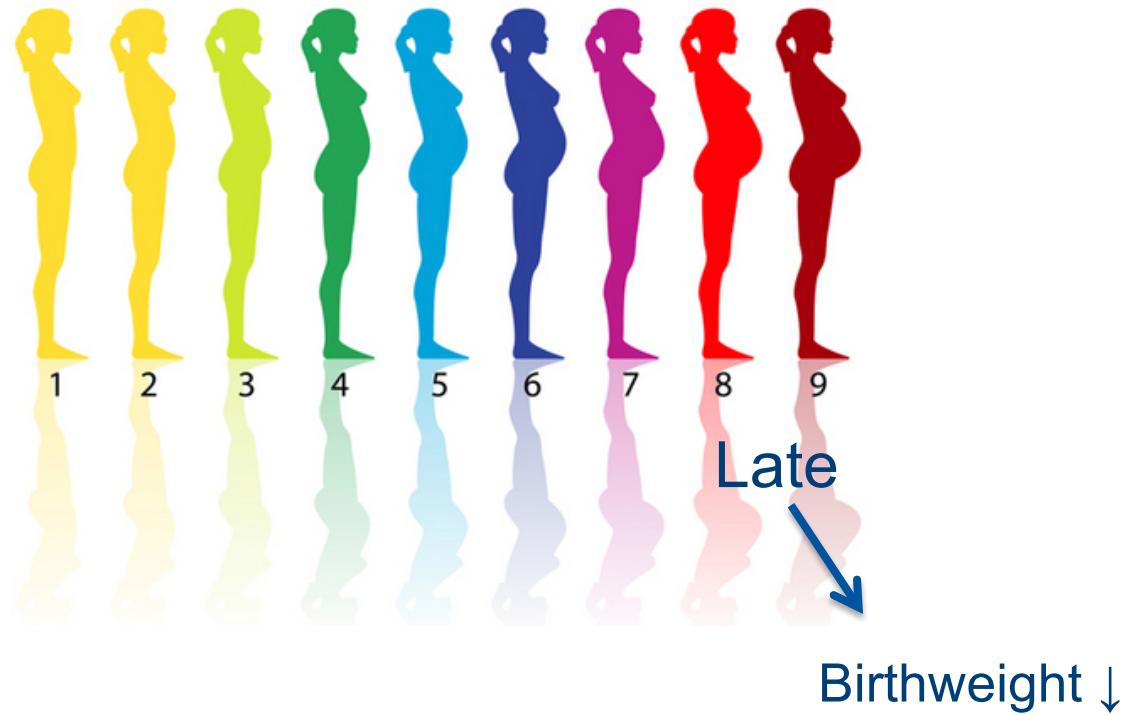
# Case: the Hungerwinter study

- Winter 1944-1945
  - Early winter
  - No supply of food and fuel  
(punishment for help during Market Garden)
  - 3.5 million people
    - Eating flower bulbs
    - Burning sleepers
  - 20.000 people died from starvation and cold



# Case: the Hungerwinter study

## Hungerwinter babies vs. siblings



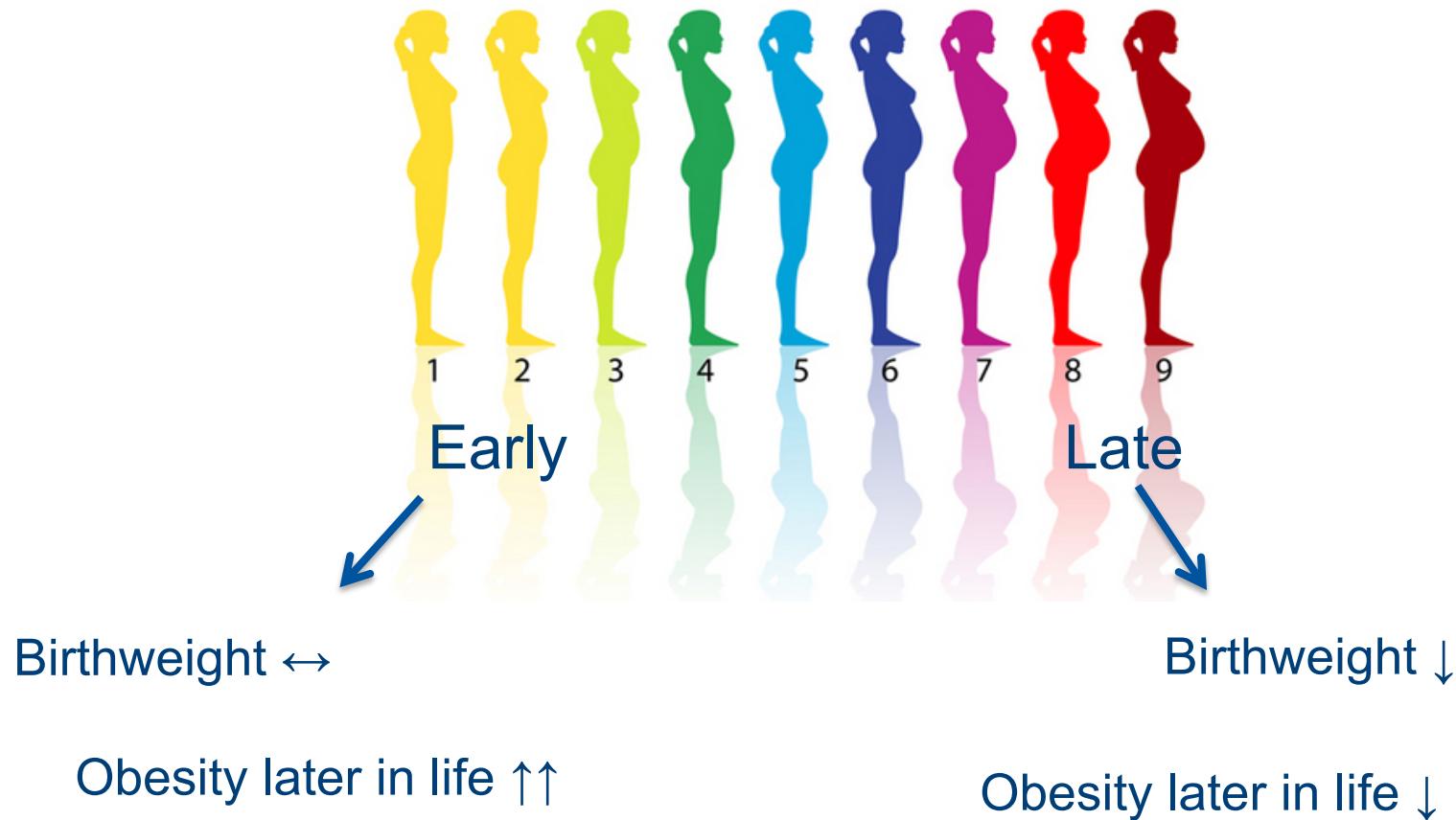
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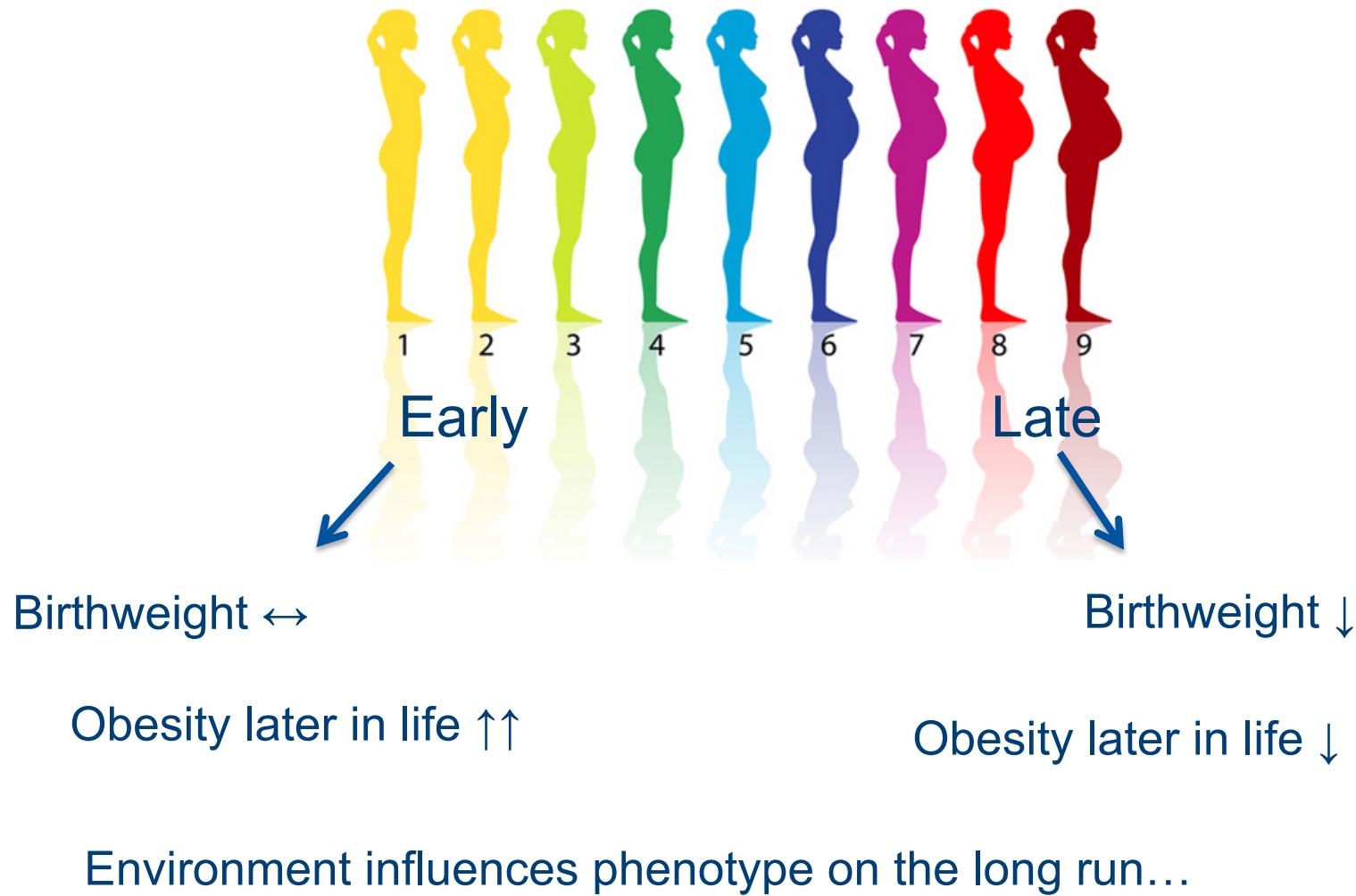
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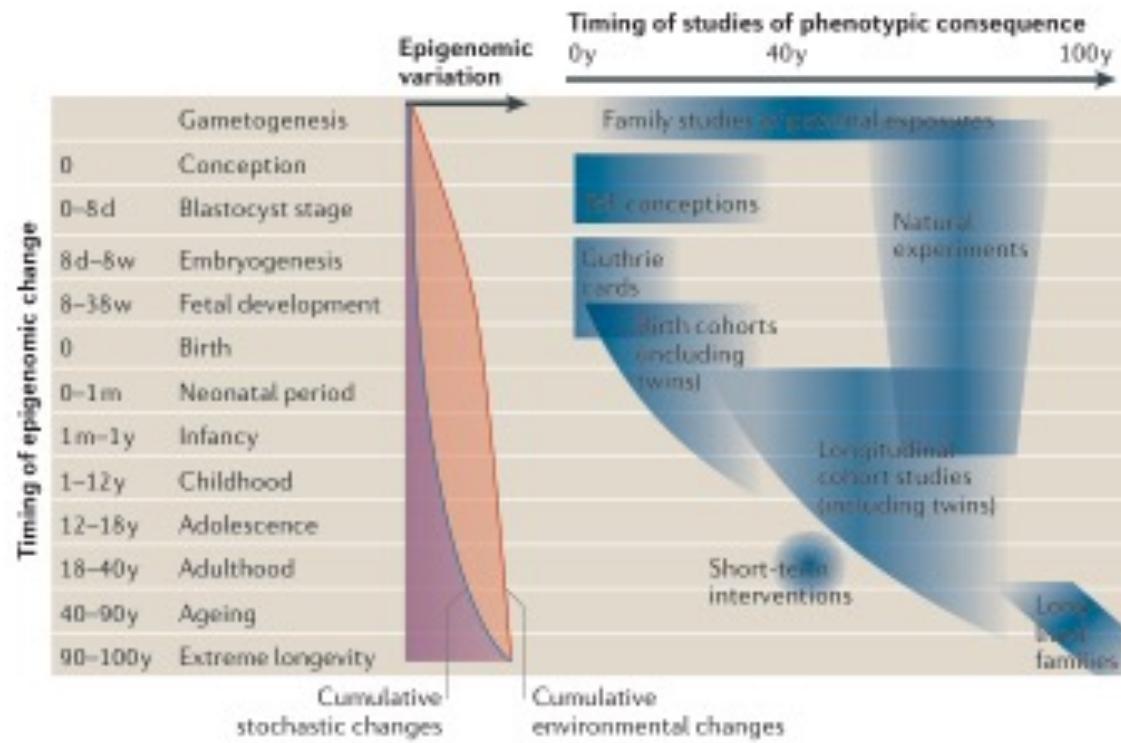
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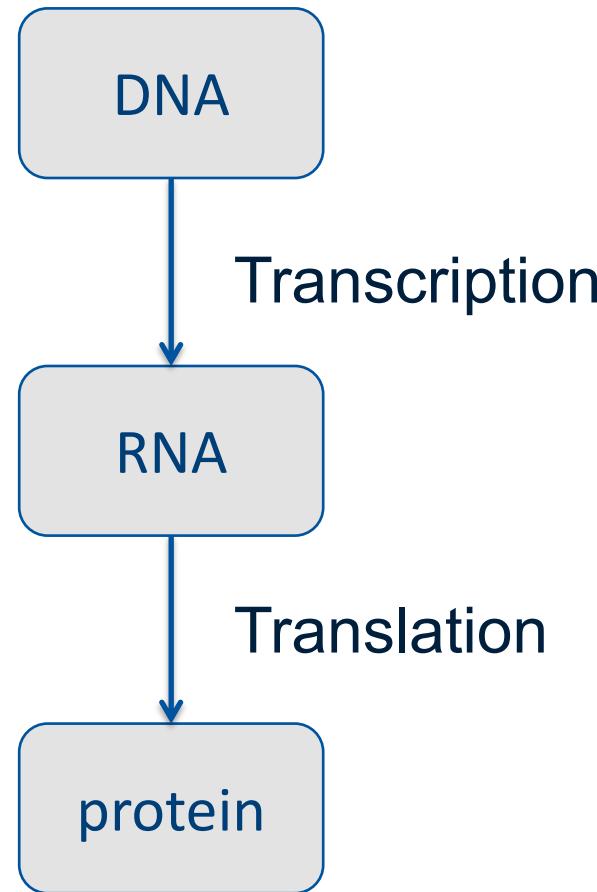


# Epigenetics

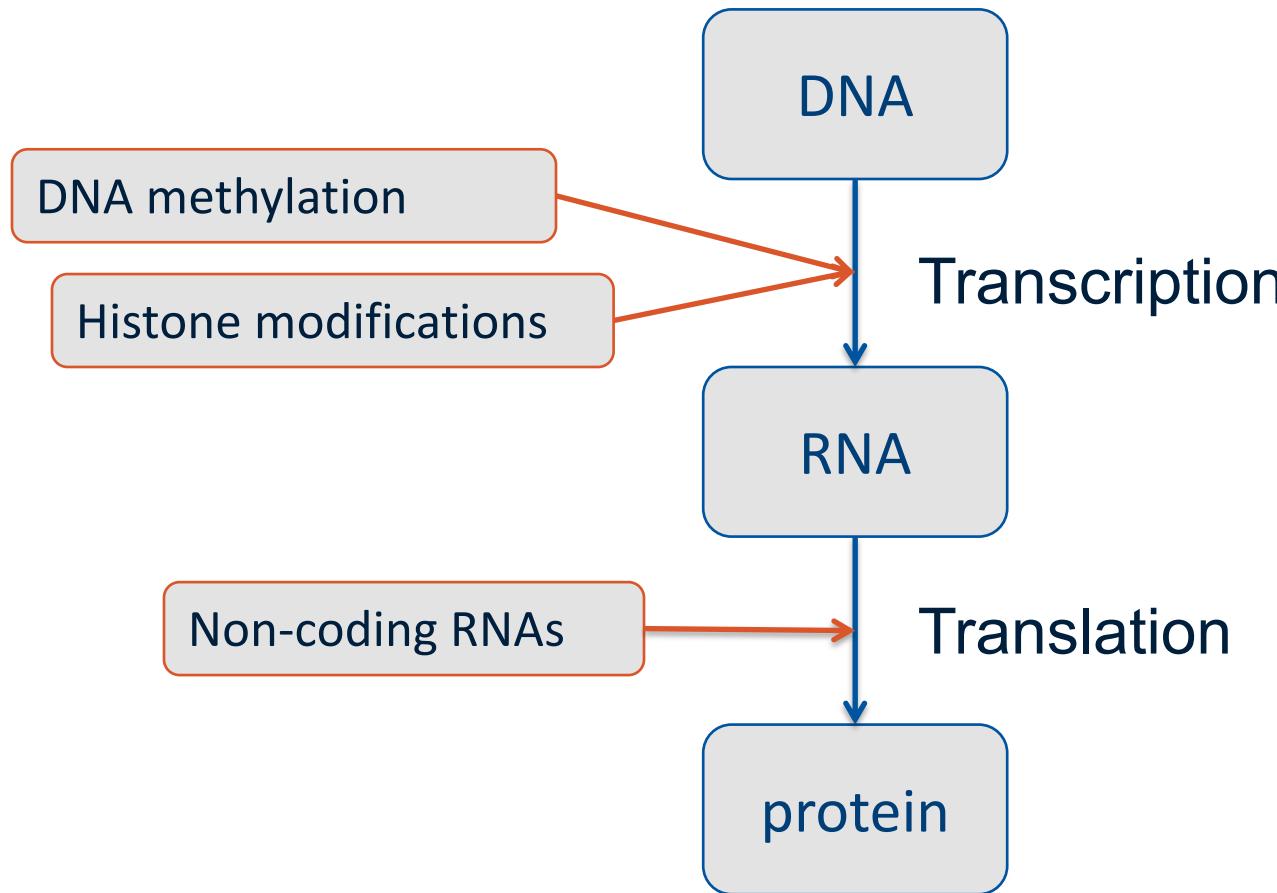
*Heritable* variation [in gene expression] that is not based on DNA sequence variation



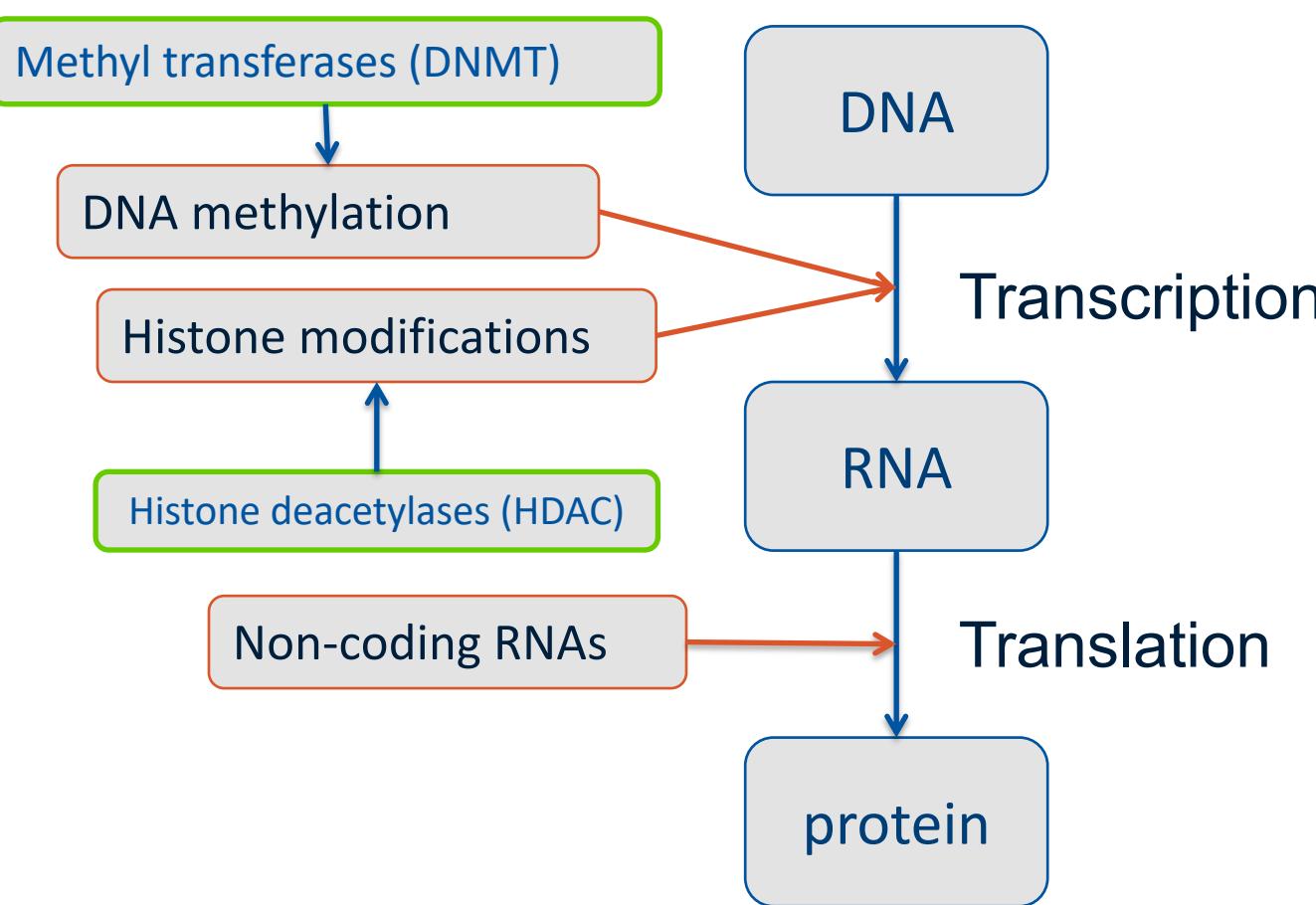
# Regulating the regulation of regulatory regulators...



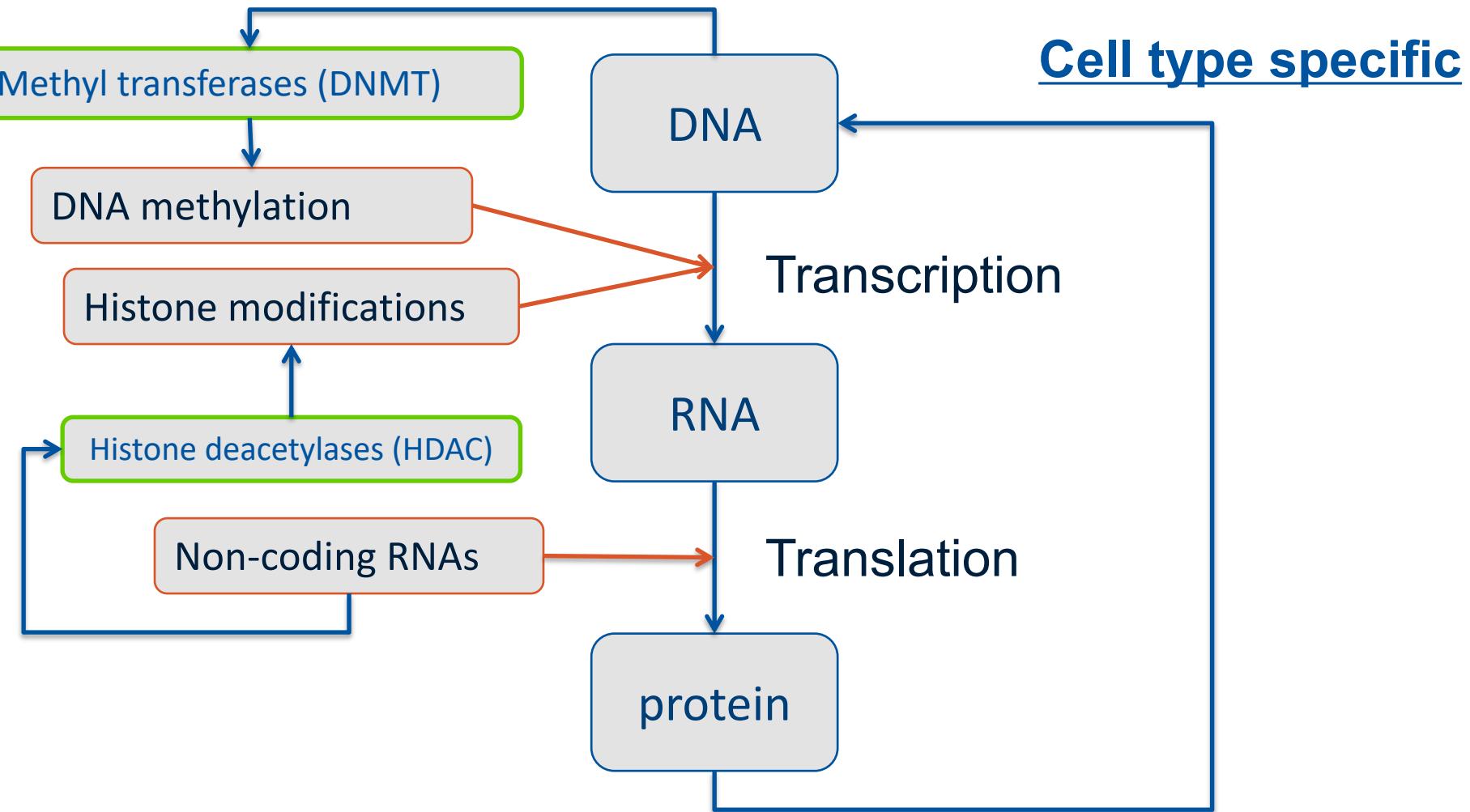
# Regulating the regulation of regulatory regulators...



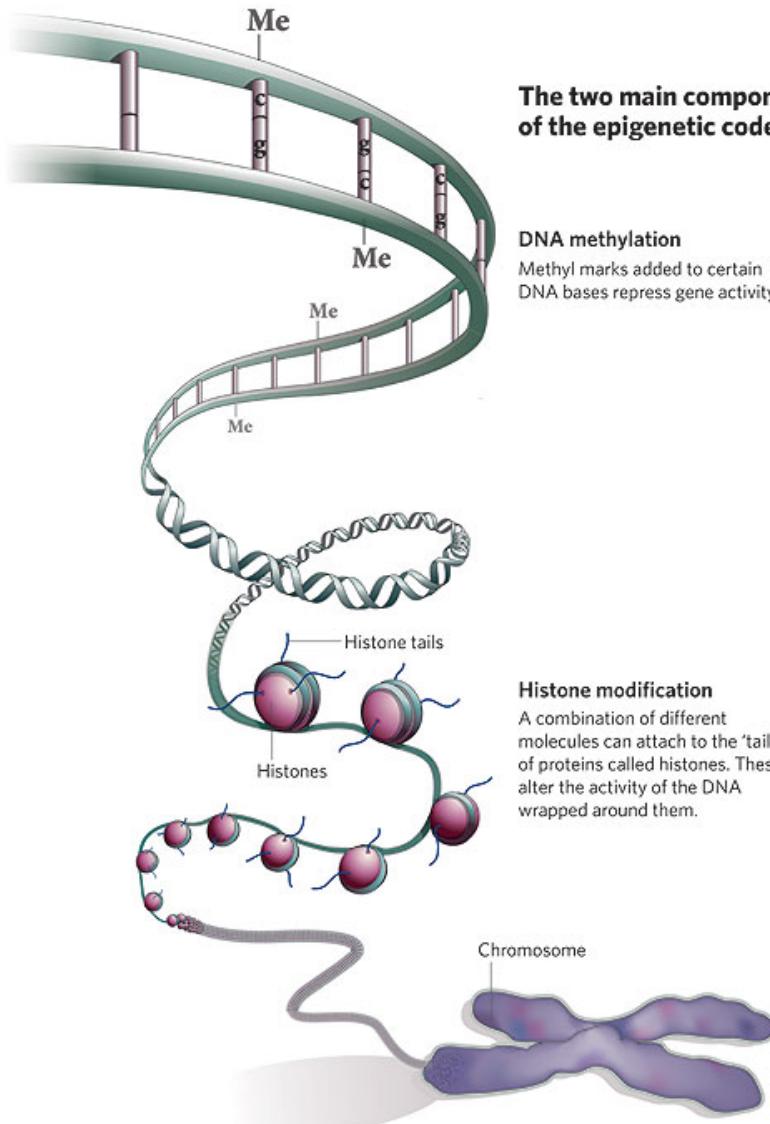
# Regulating the regulation of regulatory regulators...



# Regulating the regulation of regulatory regulators...



# The epigenetic code



**The two main components  
of the epigenetic code**

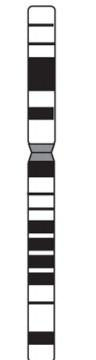
### DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

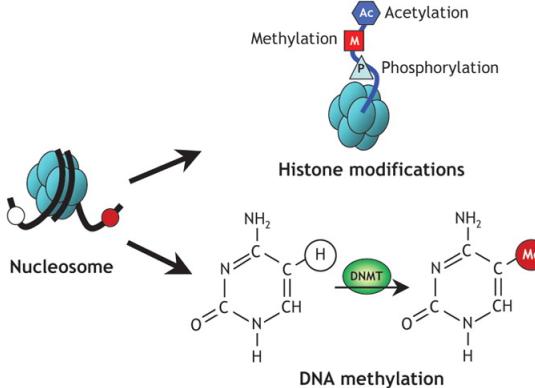
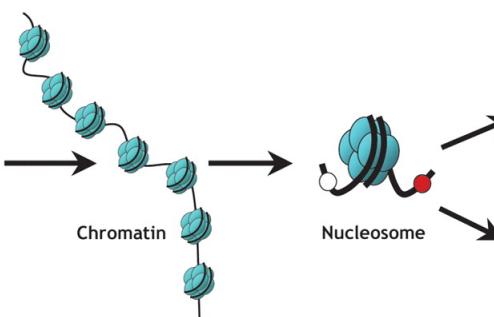
## Histone modification

A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

A



Chron



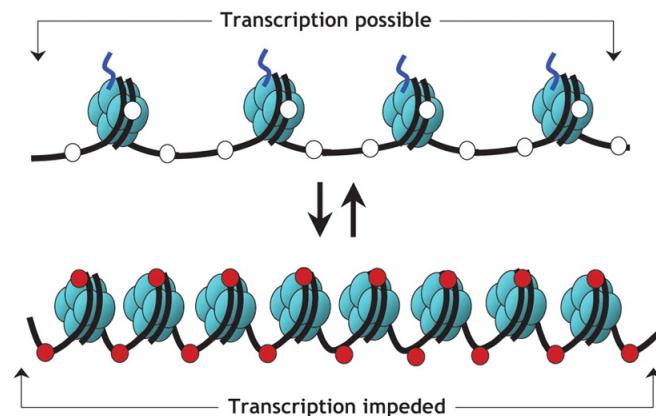
B

Gene “switched on”

- Active (open) chromatin
  - Unmethylated cytosines  
(white circles)
  - Acetylated histones

Gene “switched off”

- Silent (condensed) chromatin
  - Methylated cytosines  
(red circles)
  - Deacetylated histones



# Another \*WAS: Epigenome-wide association study of BMI

- Dick *et al.* Lancet, 2014.
- 3 epigenetic loci associated to variability in BMI
- Replicated results
- Non-causal effect: Mendelian Randomization

Articles

## DNA methylation and body-mass index: a genome-wide analysis

Katherine J Dick, Christopher P Nelson, Loukia Tsapraili, Johanna K Sandling, Dylan Aliss, Simone Wahl, Eshwar Meduri, Pierre-Emmanuel Morange, France Gagnon, Harold Grallert, Melanie Waldenberger, Annette Peters, Jeanette Erdmann, Christian Hengstenberg, Francois Cambien, Alison H Goodall, Willem H Ouwehand, Heribert Schunkert, John R Thompson, Tim D Spector, Christian Gieger, David-Alcandre Tréguer, Panos Deloukas, Nilesh J Samani



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**Methods** 479 individuals of European origin recruited by the Cardiogenics Consortium formed our discovery cohort. We typed their whole-blood DNA with the Infinium HumanMethylation450 array. After quality control, methylation levels were tested for association with BMI. Methylation sites showing an association with BMI at a false discovery rate q value of 0.05 or less were taken forward for replication in a cohort of 339 unrelated white patients of northern European origin from the MARTHA cohort. Sites that remained significant in this primary replication cohort were tested in a second replication cohort of 1789 white patients of European origin from the KORA cohort. We examined whether methylation levels at identified sites also showed an association with BMI in DNA from adipose tissue ( $n=635$ ) and skin ( $n=395$ ) obtained from white female individuals participating in the MuTHER study. Finally, we examined the association of methylation at BMI-associated sites with genetic variants and with gene expression.

**Findings** 20 individuals from the discovery cohort were excluded from analyses after quality-control checks, leaving 459 participants. After adjustment for covariates, we identified an association ( $q$  value  $\leq 0.05$ ) between methylation at five probes across three different genes and BMI. The associations with three of these probes—cg22891070, cg27146050, and cg16672562, all of which are in intron 1 of *HIF3A*—were confirmed in both the primary and second replication cohorts. For every 0.1 increase in methylation  $\beta$  value at cg22891070, BMI was 3.6% (95% CI 2.4–4.9) higher in the discovery cohort, 2.7% (1.2–4.2) higher in the primary replication cohort, and 0.8% (0.2–1.4) higher in the second replication cohort. For the MuTHER cohort, methylation at cg22891070 was associated with BMI in adipose tissue ( $p=1.72 \times 10^{-5}$ ) but not in skin ( $p=0.882$ ). We observed a significant inverse correlation ( $p=0.005$ ) between methylation at cg22891070 and expression of one *HIF3A* gene-expression probe in adipose tissue. Two single nucleotide polymorphisms—rs8102595 and rs3826795—had independent associations with methylation at cg22891070 in all cohorts. However, these single nucleotide polymorphisms were not significantly associated with BMI.

**Interpretation** Increased BMI in adults of European origin is associated with increased methylation at the *HIF3A* locus in blood cells and in adipose tissue. Our findings suggest that perturbation of hypoxia inducible transcription factor pathways could have an important role in the response to increased weight in people.

**Funding** The European Commission, National Institute for Health Research, British Heart Foundation, and Wellcome Trust.

### Introduction

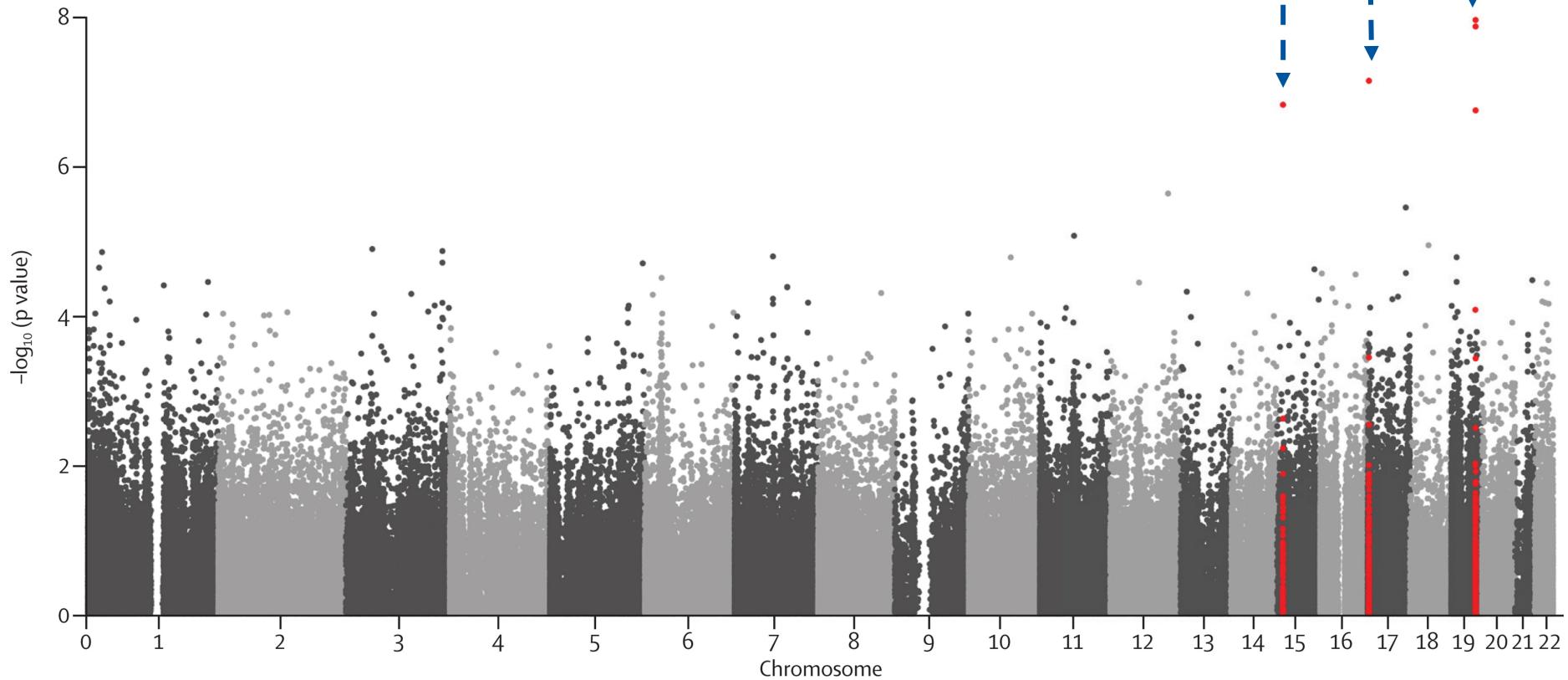
Obesity and its associated comorbidities constitute a major and growing health problem worldwide.<sup>1</sup> Therefore, understanding the mechanisms that affect body-mass index (BMI)—the most widely used measure of obesity—and any downstream effects is an important health priority. BMI is a complex phenotype determined by lifestyle (eg, physical activity), environmental factors (food availability and intake), and genetic factors.<sup>2</sup> In the past few years, a major effort to identify genetic determinants of BMI through genome-wide association studies has shown that more than 30 single nucleotide

polymorphisms (SNPs) are associated with BMI, which together explain about 1.5% of interindividual variation in BMI.<sup>3</sup>

DNA methylation is the reversible and heritable attachment of a methyl group to a nucleotide. The most common form of DNA methylation occurs at the 5' carbon of cytosine in CpG dinucleotides, creating 5-methylcytosine.<sup>4</sup> CpG dinucleotides are often located in CpG islands (clusters of CpG sites) within the promoter region or first exon of genes, or upstream from genes within CpG island shores (DNA regions within 2 kb of CpG islands) or shelves (within 2 kb of shores).<sup>5</sup> DNA

# Manhattan

HIF3A  
CLUH  
KLF13



# Main results

- “For every 10% increase in methylation of the most significant probe – cg22891070 – BMI increased by 3.6% (95% CI 2.4–4.9), equating to about 0.98kg/m<sup>2</sup> for a person in the discovery cohort with a BMI of 27 kg/m<sup>2</sup> on average.”
- “The increase in BMI was higher in individuals who had had a myocardial infarction (4.6%, 2.9–6.3) than in blood donors (2.3%, 0.4–4.1).”
- 2 genetic variants modify methylation at these sites
- Mendelian Randomization experiment: non-causal effect, *i.e.* no association of these genetic variants with BMI



# Main results

Adipose tissue (n=635)		Skin (n=395)	
p value	Percentage change in BMI*	p value	Percentage change in BMI*
cg22891070	$1.72 \times 10^{-5}$	6.2 (3.4 to 9.0)	0.882 -0.25 (-3.6 to 3.0)
cg27146050	$9.27 \times 10^{-7}$	11.9 (7.2 to 16.7)	0.011 -7.0 (-12.4 to -1.7)
cg16672562	$5.01 \times 10^{-6}$	7.9 (4.5 to 11.2)	0.862 -0.36 (-4.3 to 3.5)

Data in parentheses are 95% CIs. BMI= body-mass index. \* The  $\beta$  coefficients from the association analysis have been converted into percentage change in BMI for every 0.1 unit increase in methylation  $\beta$  value.

**Table 3:** Association between BMI and methylation at sites in HIF3A in adipose tissue and skin DNA in the MuTHER cohort



# Main results

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	rs8102595			rs3826795		
	Frequency of effect allele*	$\beta$ (95% CI)	p value	Frequency of effect allele†	$\beta$ (95% CI)	p value
Discovery (Cardiogenics)	0.10	0.063 (0.042-0.083)	$6.29 \times 10^{-3}$	0.81	0.039 (0.023-0.056)	$3.21 \times 10^{-6}$
Primary replication cohort (MARTHA)	0.10	0.097 (0.062-0.121)	$1.41 \times 10^{-3}$	0.79	0.051 (0.023-0.076)	$2.14 \times 10^{-5}$
Second replication cohort (KORA)	0.09	0.073 (0.058-0.086)	$9.18 \times 10^{-22}$	0.82	0.048 (0.037-0.059)	$2.26 \times 10^{-18}$
MuTHER cohort: adipose tissue	0.10	0.041 (0.033-0.049)	$1.05 \times 10^{-21}$	0.81	0.021 (0.014-0.028)	$3.61 \times 10^{-9}$
MuTHER cohort: skin	0.10	0.062 (0.052-0.074)	$7.09 \times 10^{-25}$	0.82	0.023 (0.013-0.034)	$1.77 \times 10^{-5}$

The  $\beta$  values are from an additive model and are a unit change in methylation per copy of the effect allele. \*G. †C.

**Table 4:** Association between methylation level at cg22891070 and single nucleotide polymorphisms at the HIF3A locus

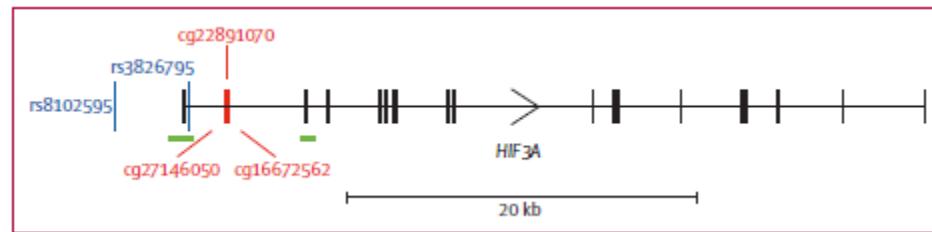


# Main results

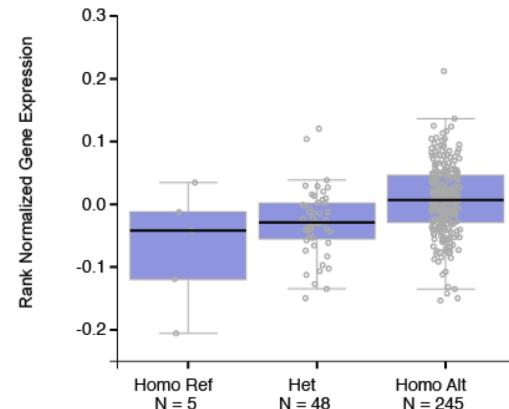
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rs8102595 vs. HIF3A (ENSG00000124440.11) in adipose subcutaneous tissue



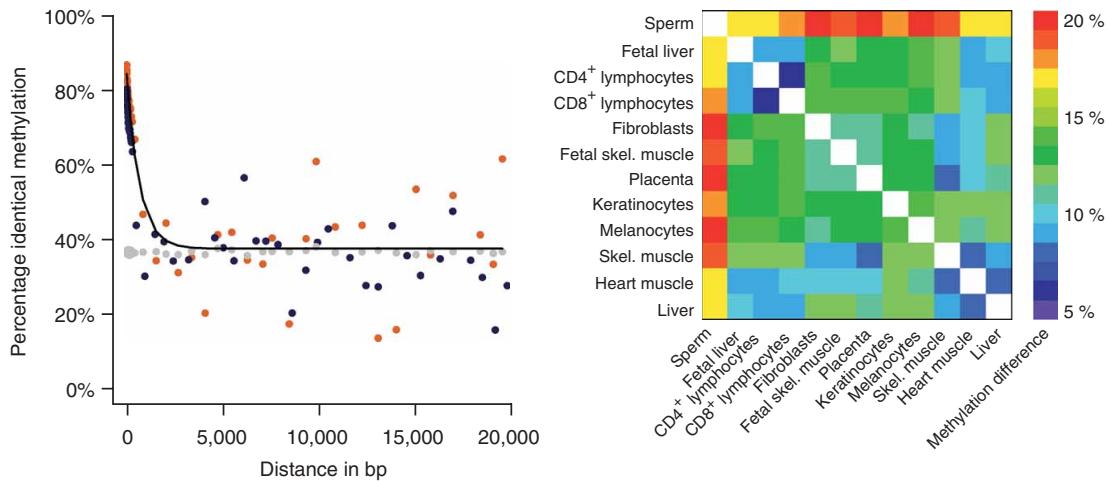
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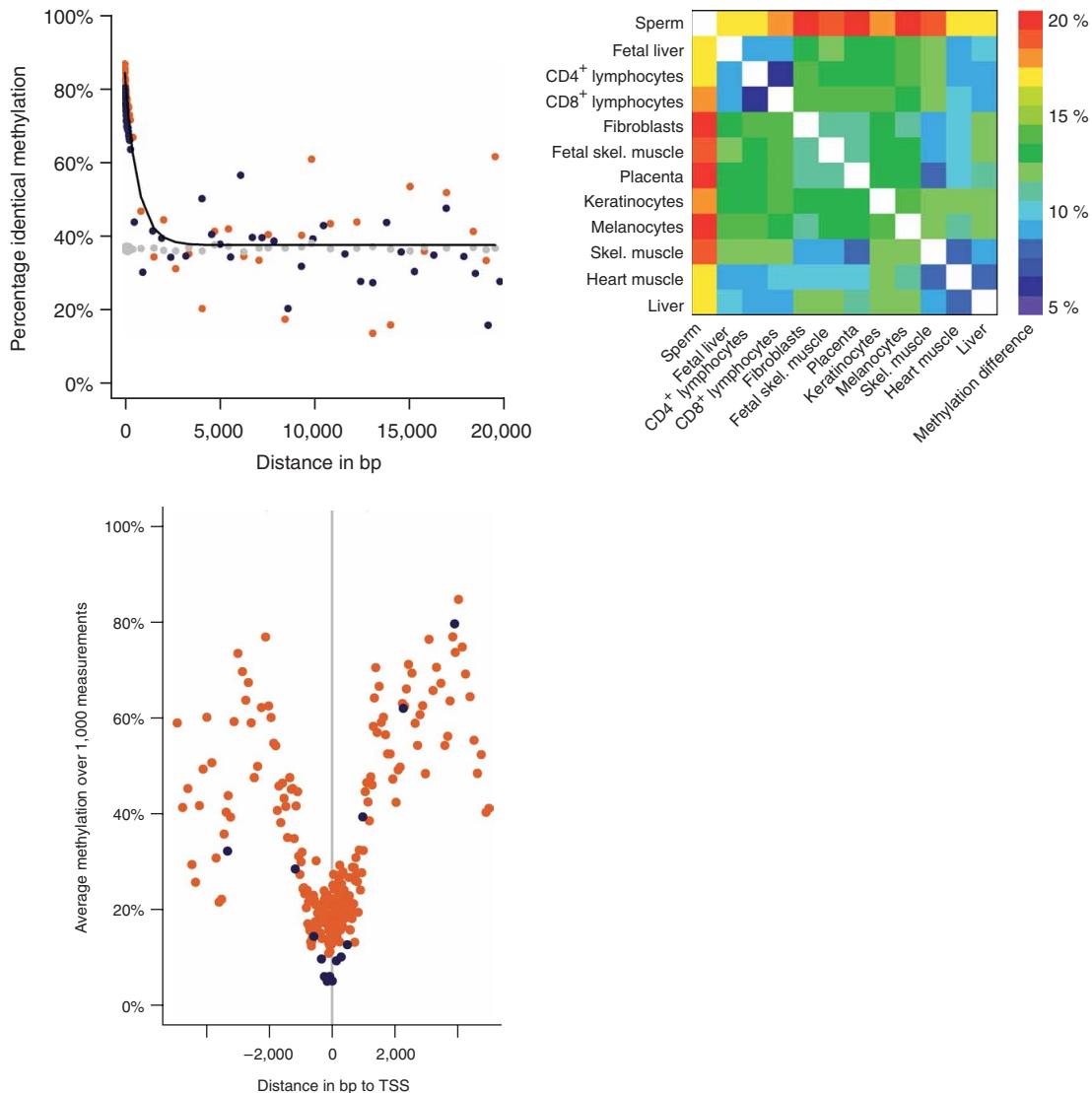
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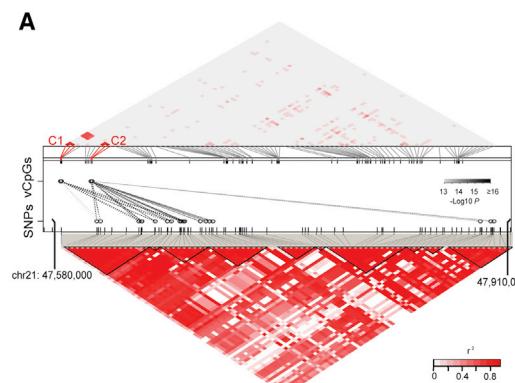
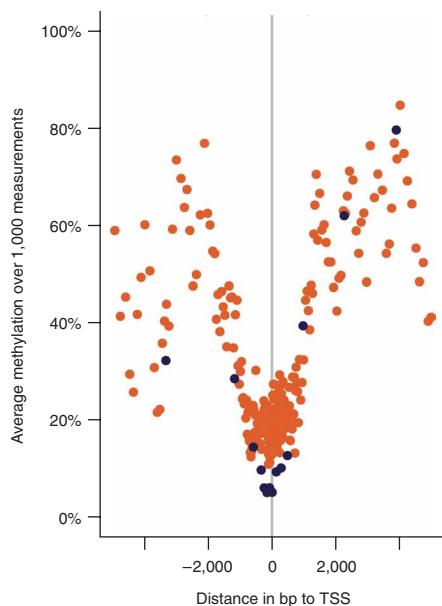
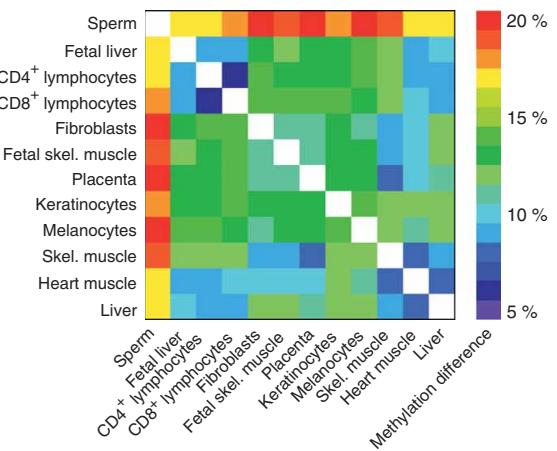
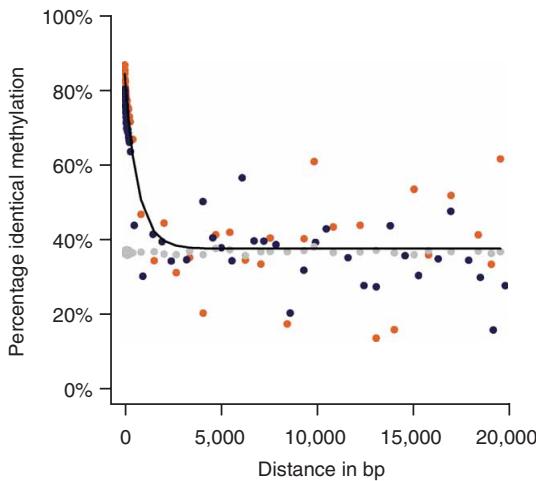
# CpGs aren't SNPs



# CpGs aren't SNPs



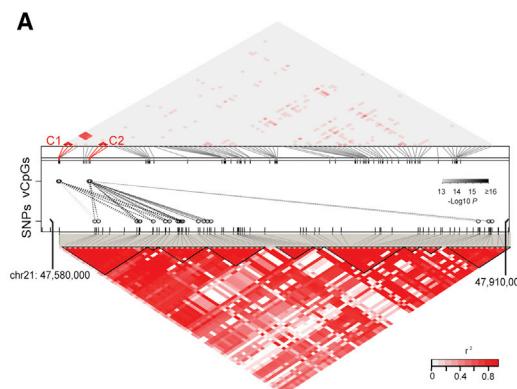
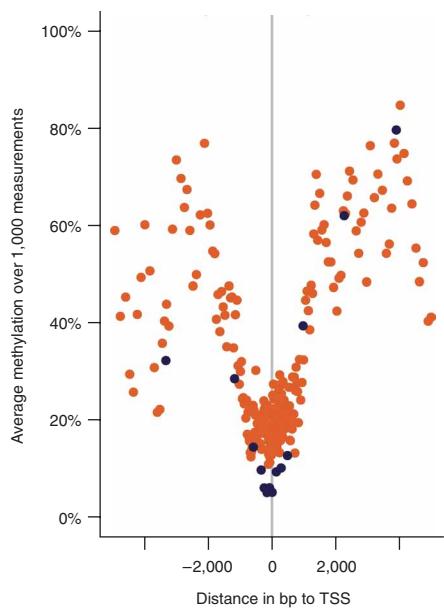
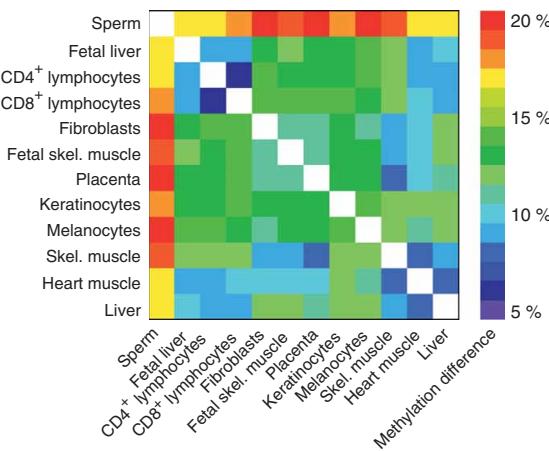
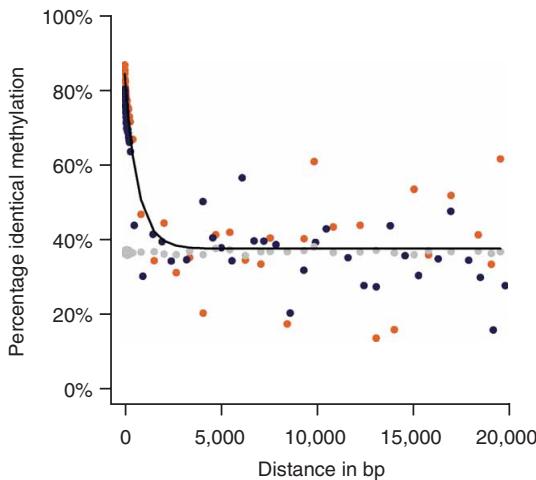
# CpGs aren't SNPs



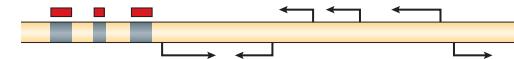
Eckhardt F. et al. *Nature Genetics*; 38:12; 2006  
 Suzuki MM. et al. *Nature Reviews Genetics*; 9; 2008  
 Liu Y. et al. *AHJG*; 94; 2014



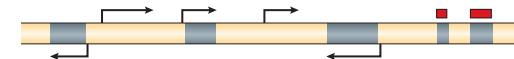
# CpGs aren't SNPs



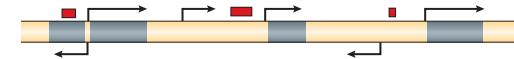
**a Mosaic DNA methylation**  
(fungi, for example, *Neurospora crassa*)



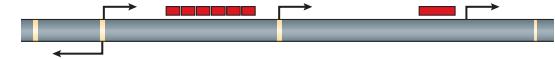
**b Mosaic DNA methylation**  
(plants, for example, *Arabidopsis thaliana*)



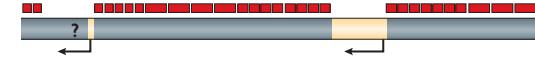
**c Mosaic DNA methylation**  
(animals, for example, *Ciona intestinalis*)



**d Global DNA methylation**  
(animals, for example, *Homo sapiens*)



**e Global DNA methylation**  
(plants, for example, *Zea mays*)





A tip of the veil

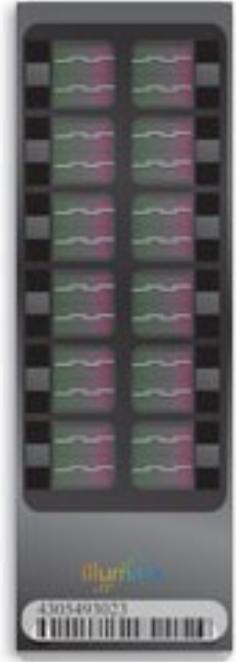
# OUR RESEARCH



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# Methylomics in the AE

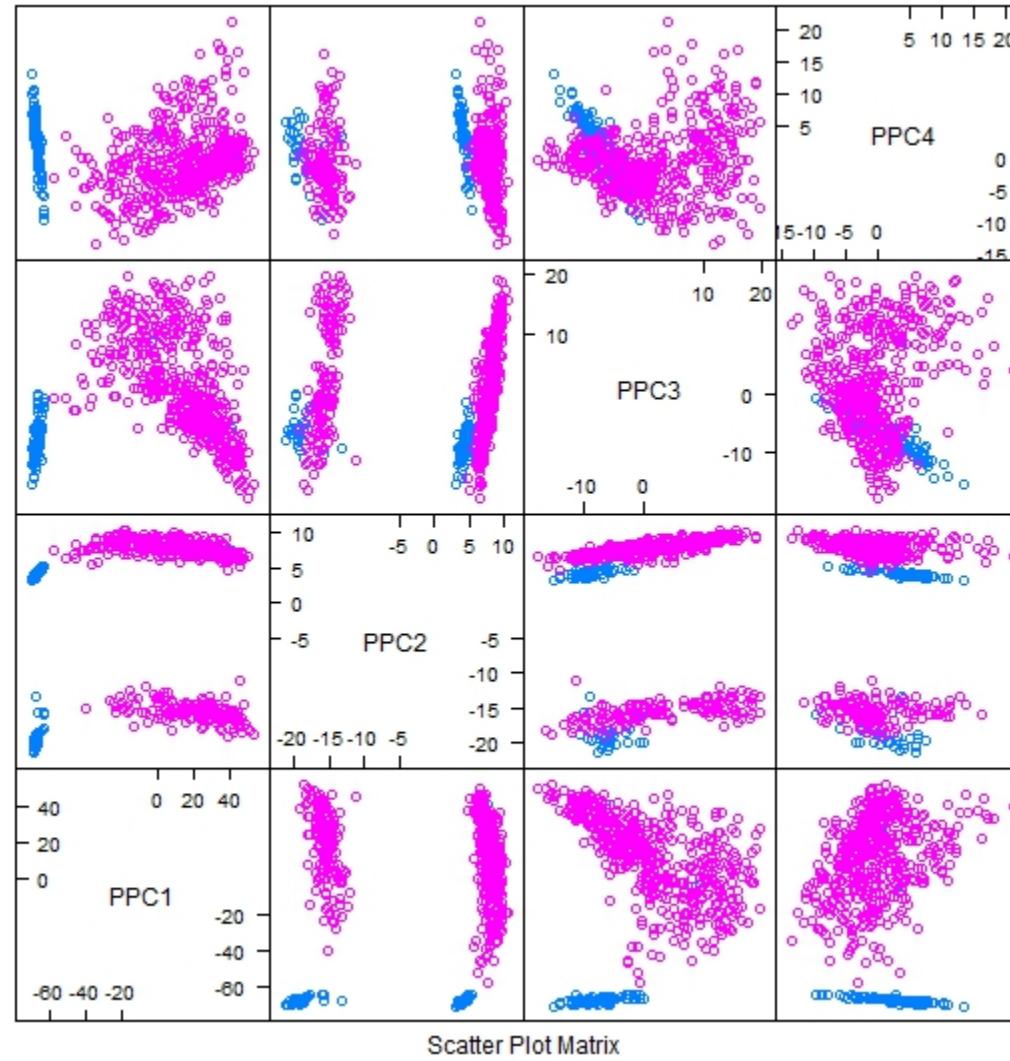
- Only CEA patients, ~95% genotyped
- Illumina HumanMethylation450K
  - 450K CpG probes
- Athero-Express Methylomics Study 1 (AEMS450K1)
  - 487 plaque samples
  - 91 blood samples
- Athero-Express Methylomics Study 2 (AEMS450K2)
  - ~400 plaque samples



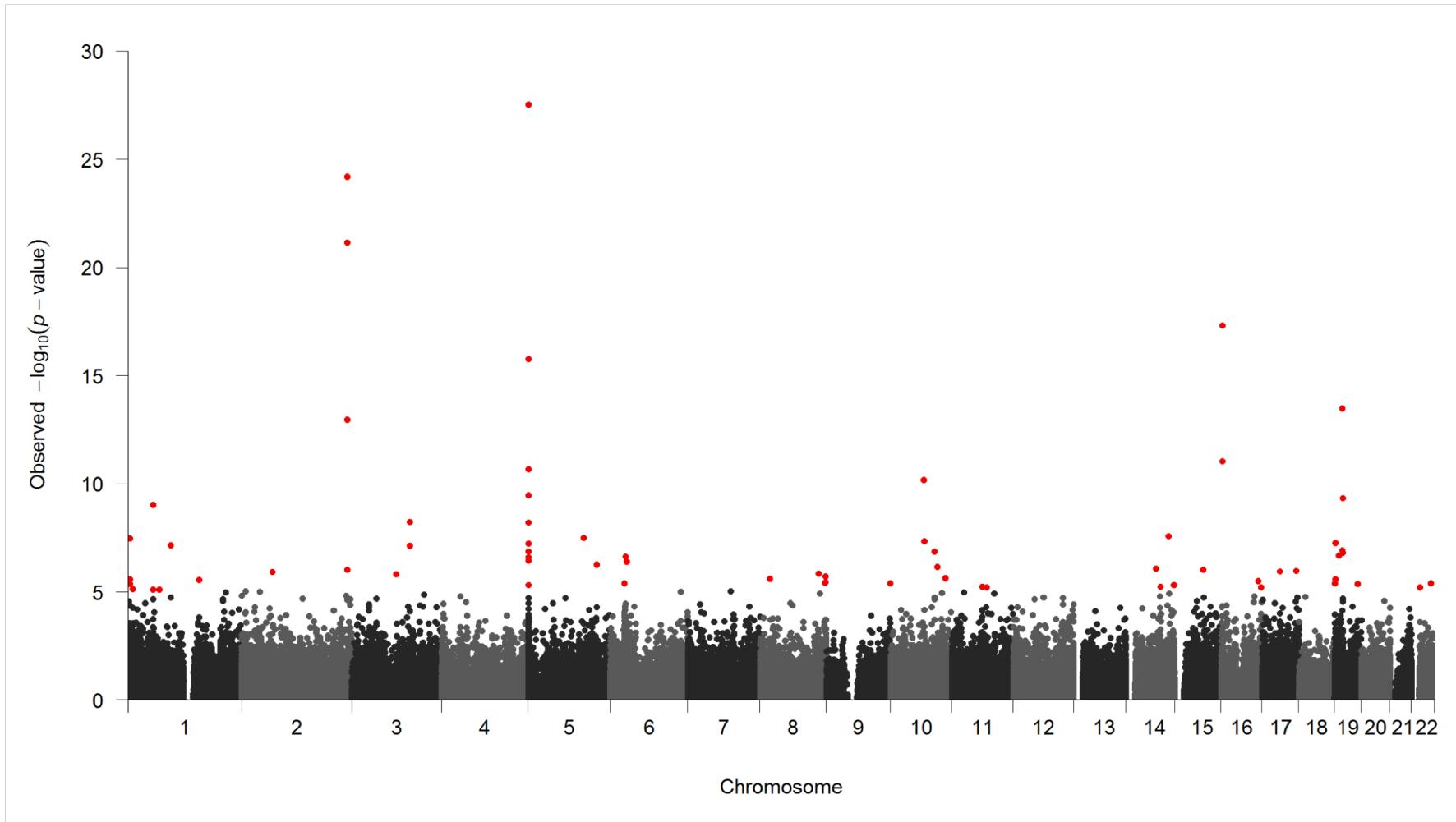
Illumina HM 450K:  
450.000 loci



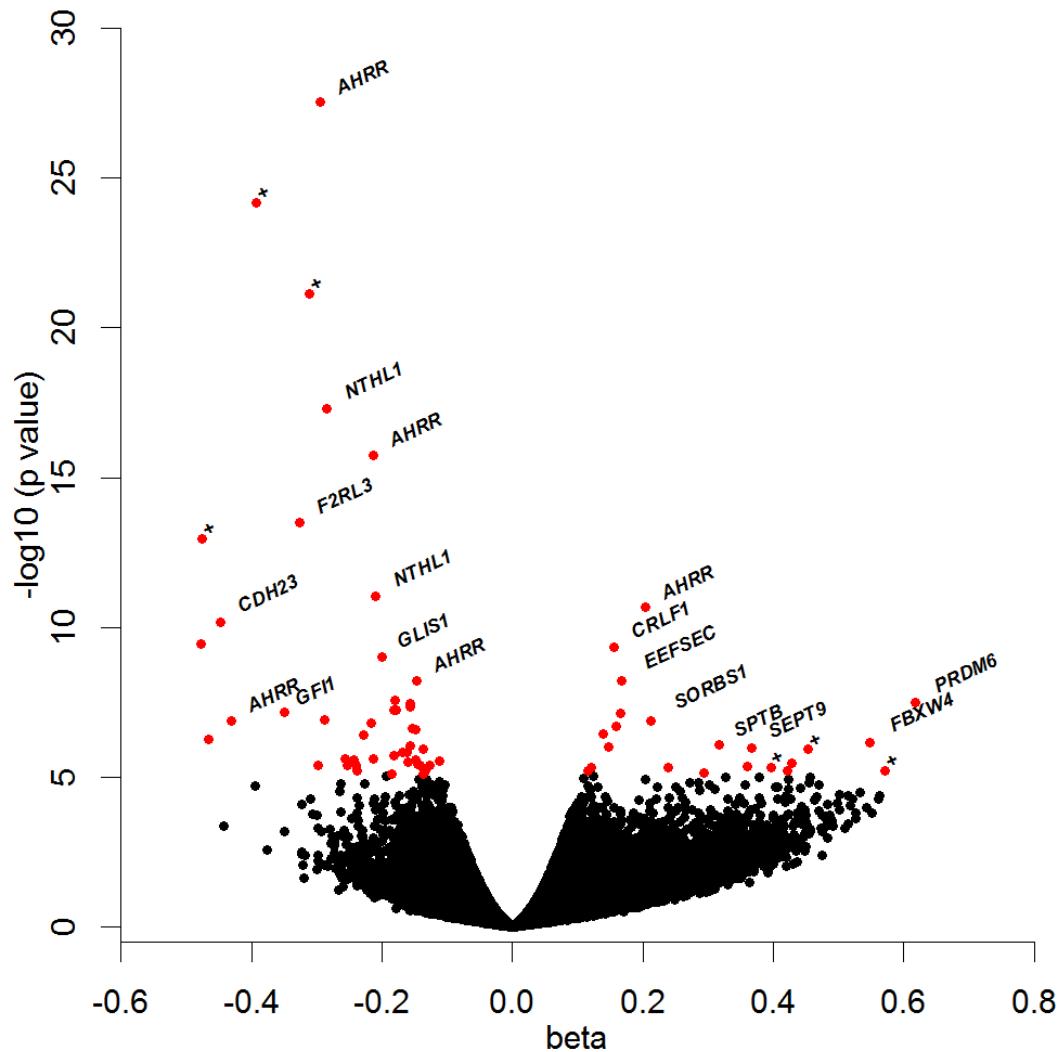
# Methylation differs between blood and plaques



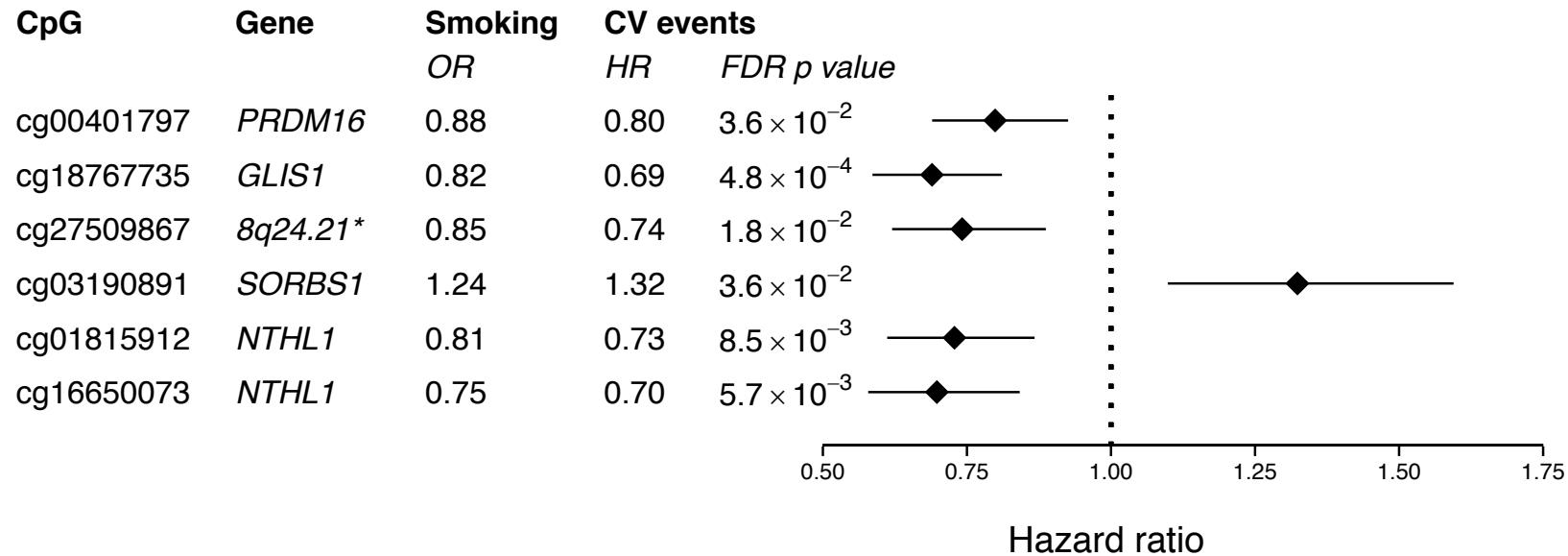
# What is the impact of smoking on the arterial wall?



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# Cardiovascular Genetic Research

## Laboratory of Experimental Cardiology

Dr. Sander W. van der Laan

### Cardiology

Prof. Dr. F.W. Asselbergs

Dr. Jessica van Setten

Dr. Magdalena Harakalova

### Research topics

Biomarker Discovery & Validation

*Athero-Express | AAA-Express | CTMM*

Ischemic stroke

GWAS | 4C | CRISPR-Cas9

Cardiovascular Genomics

Next-Gen Sequencing | eQTL | pQTL |

Transcriptomics | Epigenomics

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Een privaat-publiek initiatief

