

# Integrating genetic and epigenetic variation in schizophrenia

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SCHOOL | **KING'S**  
*College*  
**LONDON**

# Integrated –omics approach: heterogeneous etiology, convergent molecular pathology

LETTER

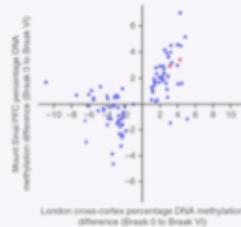
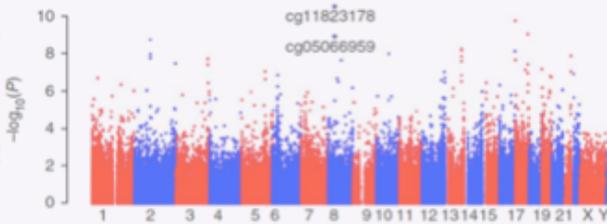
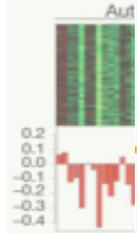
doi:10.1038/nature10110

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Methylomic profiling of human brain tissue supports a neurodevelopmental origin for

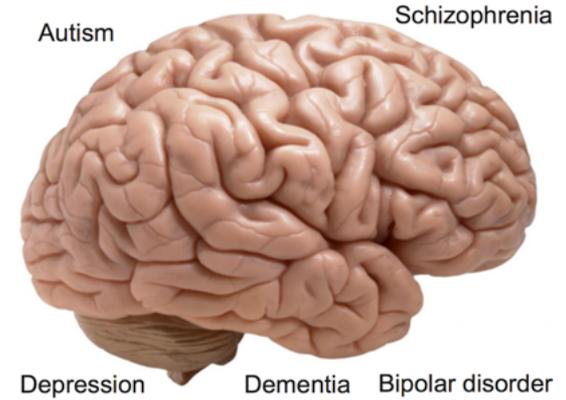
schizop

Methylomic profiling implicates cortical deregulation of *ANK1* in Alzheimer's disease



Lunnon et al, Nature Neuroscience, 2014

## Functional annotation of human brain genome



- Human brain tissue*
- Biomarkers*
- Clinical cohorts*
- Animal models*
- Cell models (iPSC)*
- Genome editing*
- Single-cell profiling*

**Genomics**  
(GWAS)

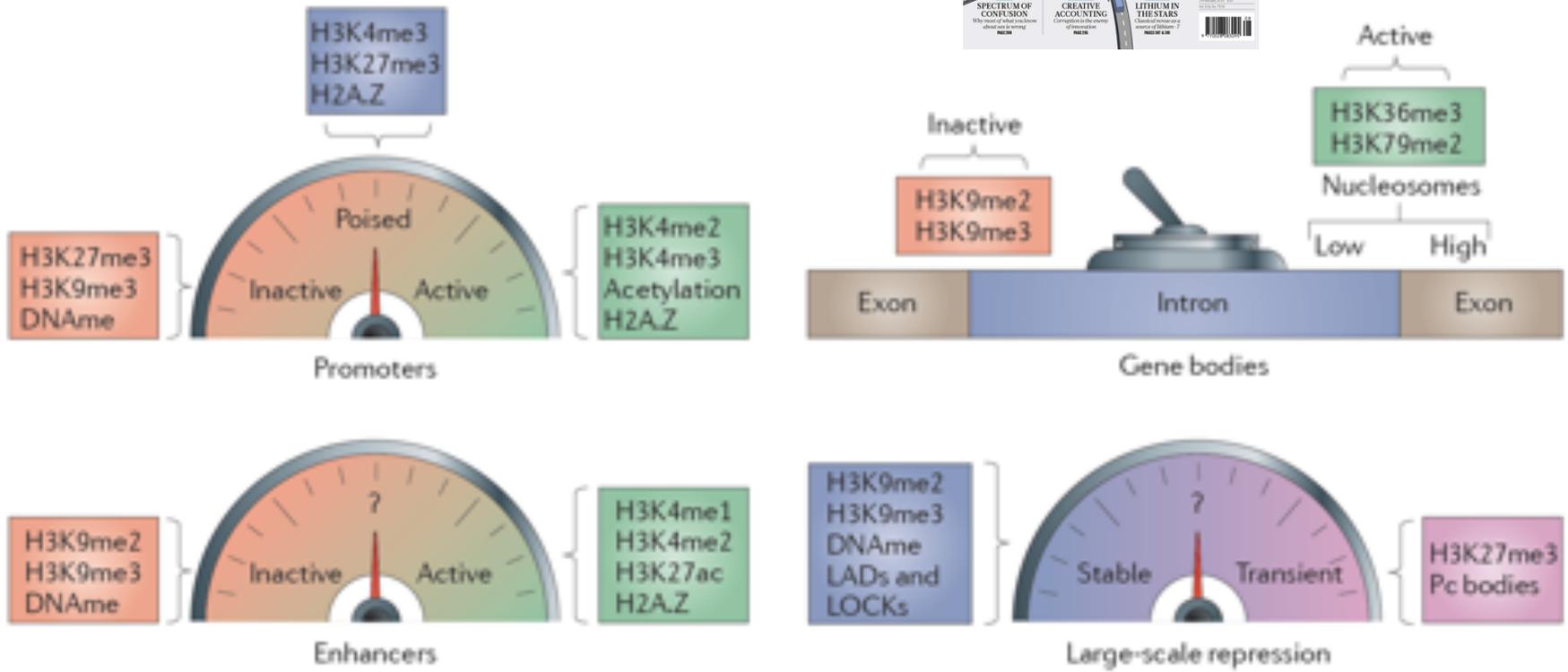
**Epigenomics**  
(5mC, 5hmC, histone modifications)

**Transcriptomics**  
(RNA-seq)



**Systems-level analyses**

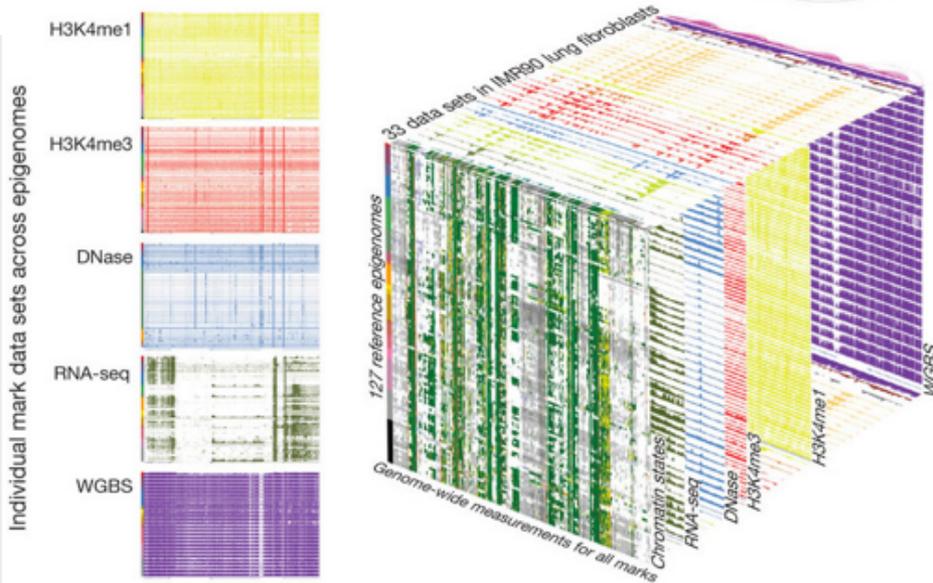
# A suite of epigenetic modifications act to fine-tune genomic function



Zhou et al (2011)

Additional DNA modifications: 5hmC, 5fC, 5cC

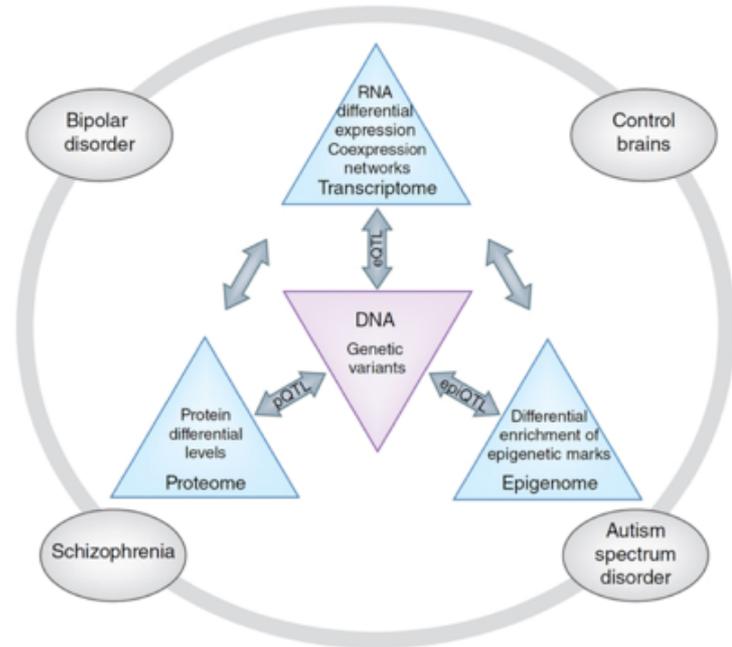
# Functional annotation of regulatory variation in the genome



Roadmap Epigenomics Consortium, Nature, 2015

# The PsychENCODE project

The PsychENCODE Consortium\*, Schahram Akbarian, Chunyu Liu, James A Knowles, Flora M Vaccarino, Peggy Farnham, Gregory E Crawford, Andrew E Jaffe, Dalila Pinto, Stella Dracheva, Daniel H Geschwind, Jonathan Mill, Angus C Nairn, Alexej Abyzov, Sirisha Pochareddy, Shyam Prabhakar, Sherman Weissman, Patrick F Sullivan, Matthew W State, Zhiping Weng, Mette A Peters, Kevin P White, Mark B Gerstein, Geetha Senthil, Thomas Lehner, Pamela Sklar & Nenad Sestan



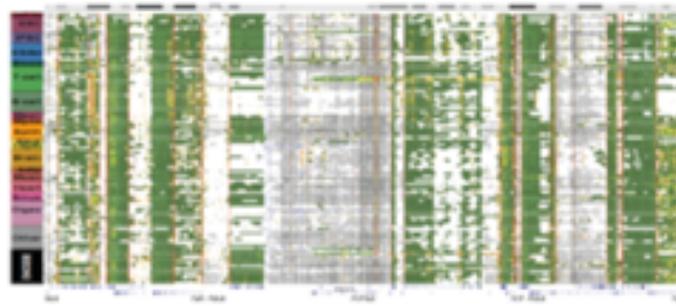
PsychENCODE Consortium, Nature Neuroscience, 2016

## Gene variants in human disease



+

## Epigenomic data for many normal human cell/tissue types



**Regulatory elements**

**Enhancers**

*Generate hypotheses about function*

# Integrated genetic-epigenetic analysis of schizophrenia

Multi-centre clinical cohorts  
UCL (n = 675)  
ABER (n = 971)  
KCL (n = 801)  
CARDIFF (n = 950)  
DUBLIN (n = 713)  
EDIN (n = 540)

SZ-  
discordant  
monozygotic  
twin pairs  
(n = 99  
pairs)

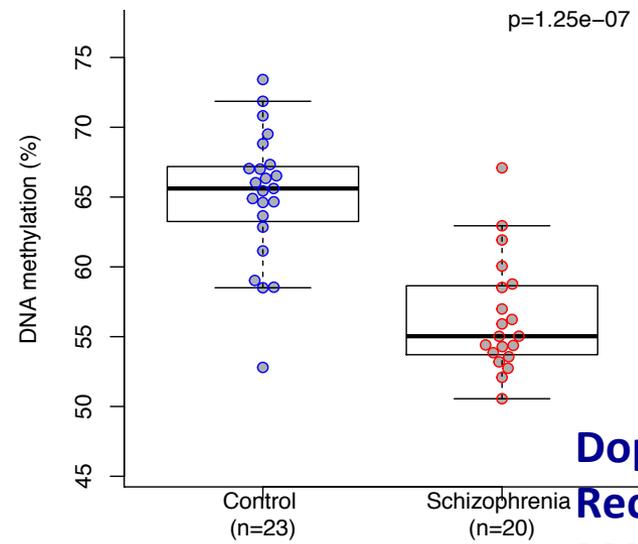
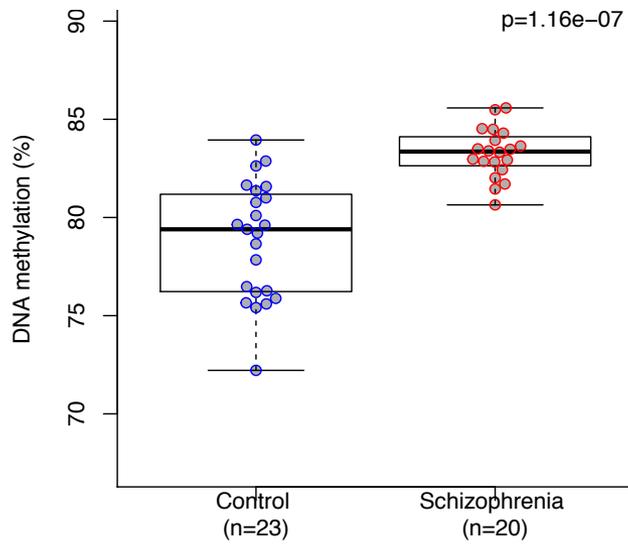
Adult post-  
mortem brain  
(matched PFC,  
striatum,  
hippocampus,  
cerebellum)

Human fetal  
brain (23 to 184  
days post-  
conception)  
(n = 179)

- Illumina 450K/EPIC DNA methylation data
- Genotype data (imputed to 1000G phase 3)

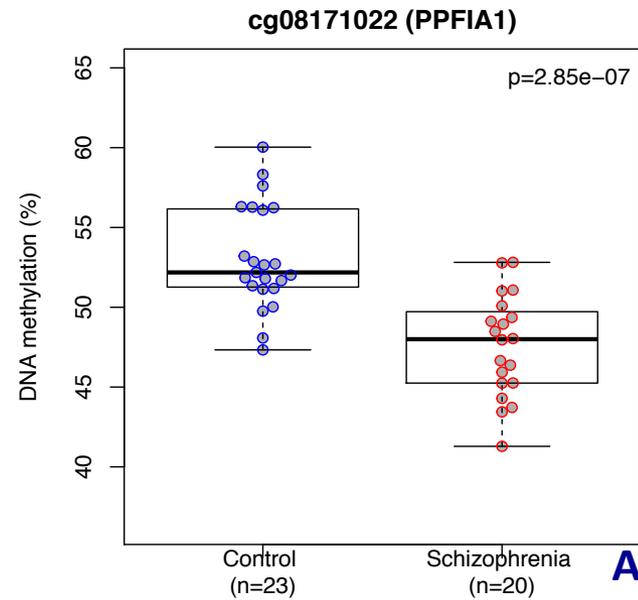
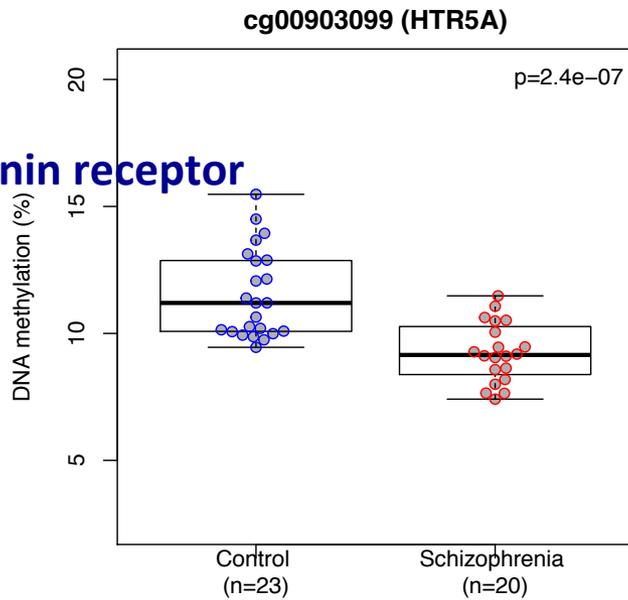
- Differences in DNA methylation associated with schizophrenia
- Differences in DNA methylation associated with high polygenic burden for schizophrenia
- Epigenetic consequences of genetic variants associated with schizophrenia

**PFC**



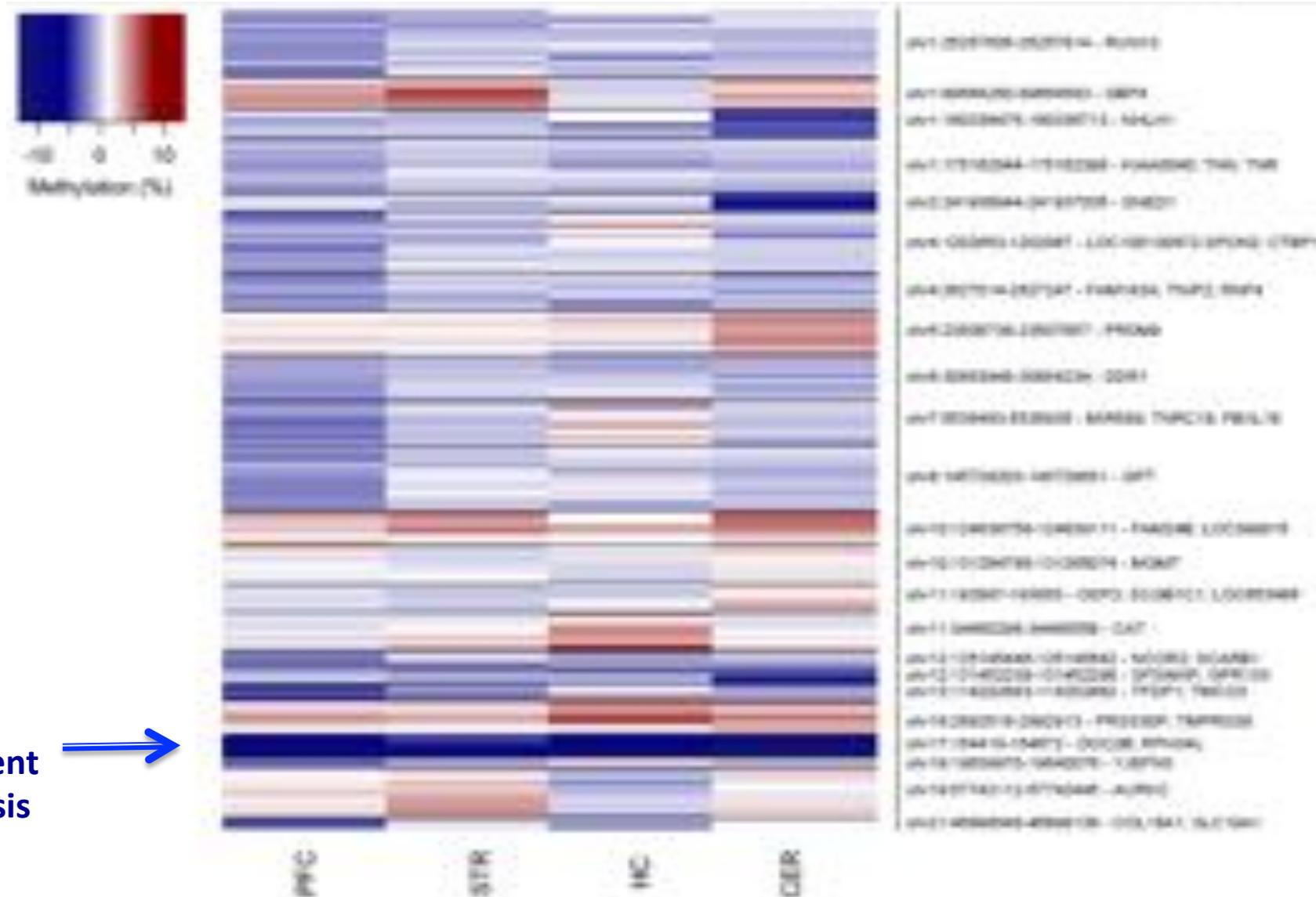
**Dopamine D2S  
Receptor-mediated  
MAPK signaling**

**Serotonin receptor**



**Axon guidance**

# Schizophrenia-associated differentially methylated regions – consistent signals across different brain regions



**RPH3AL:**  
calcium-  
dependent  
exocytosis



# Biological insights from 108 schizophrenia-associated genetic loci

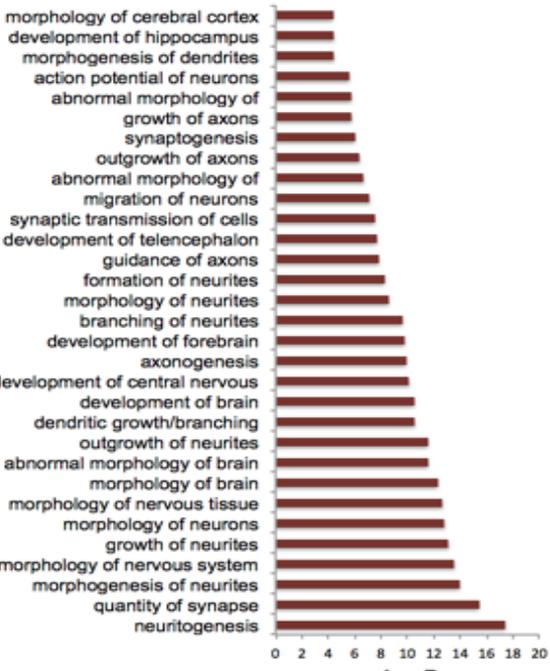
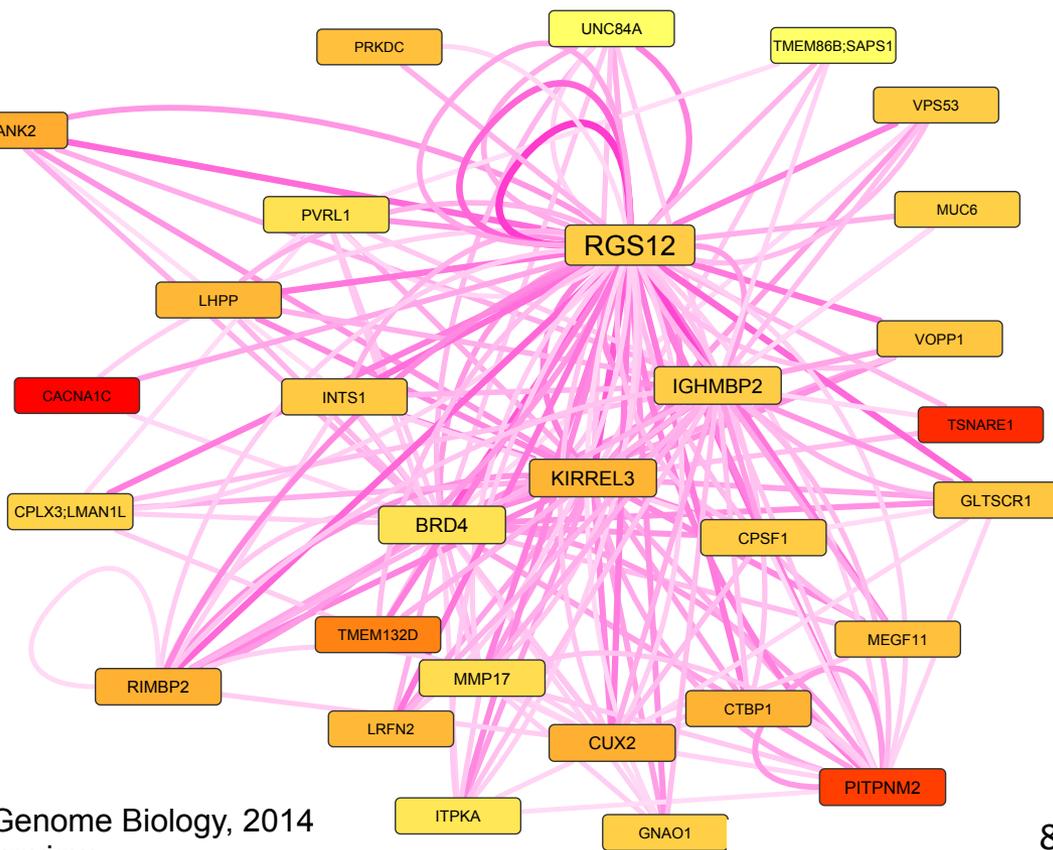
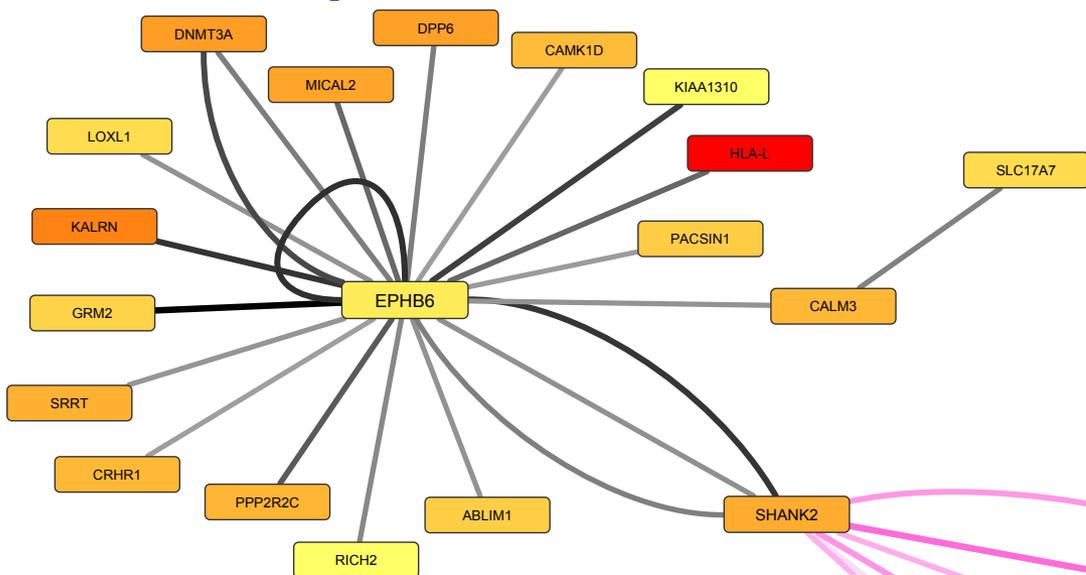
Schizophrenia Working Group of the Psychiatric Genomics Consortium\*

Key for node colour



\*best p-value within 5kb of gene coding region in largest GWAS to date (Schizophrenia Working Group of the Psychiatric Genomics Consortium *Nature* 2014)

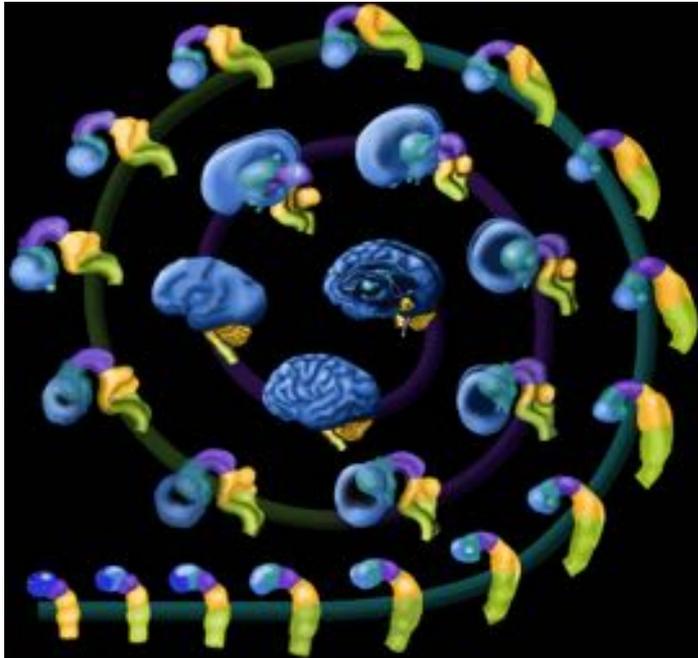
# PFC co-methylated modules associated with schizophrenia



Pidsley et al, *Genome Biology*, 2014  
 Viana et al, in review

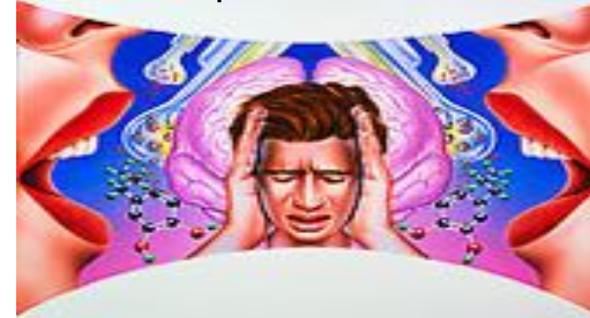
# Neurodevelopmental origins of mental illness

## Normal Brain Development



*Mutations*  
*Polygenic variation*  
*Environmental insults*  
*Stochastic factors*

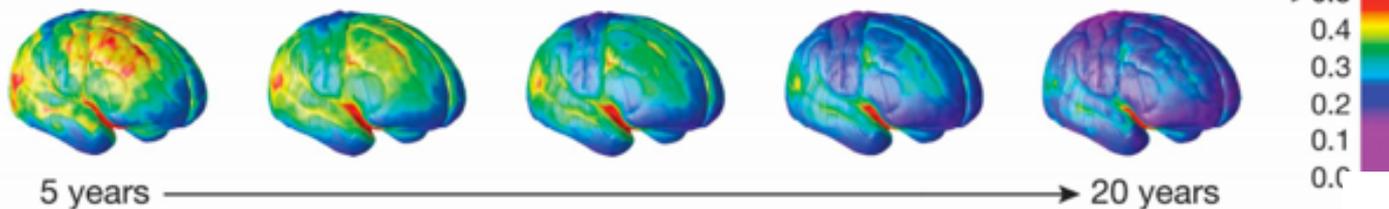
## Schizophrenia



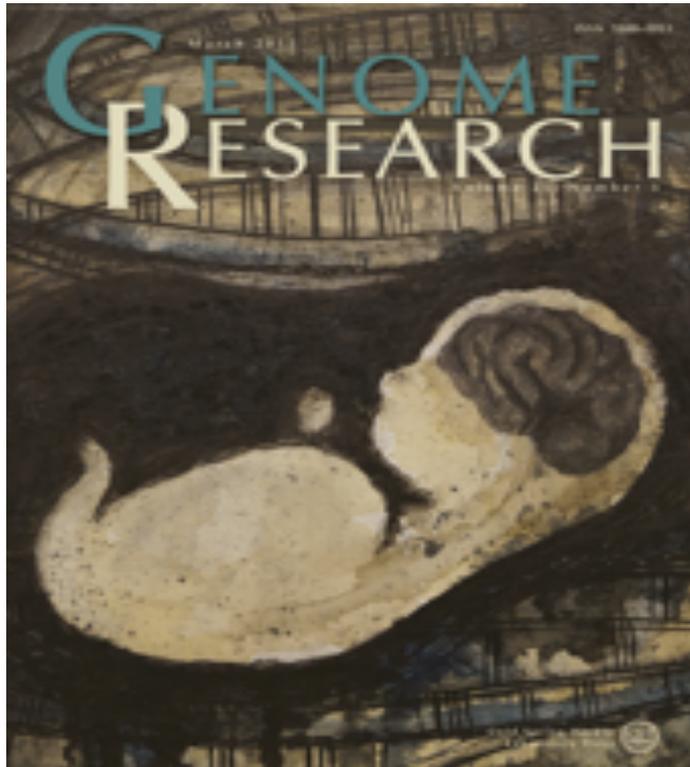
## Autism



Gray matter volume changes during normal development



# Fetal brain tissue from 191 elective abortions



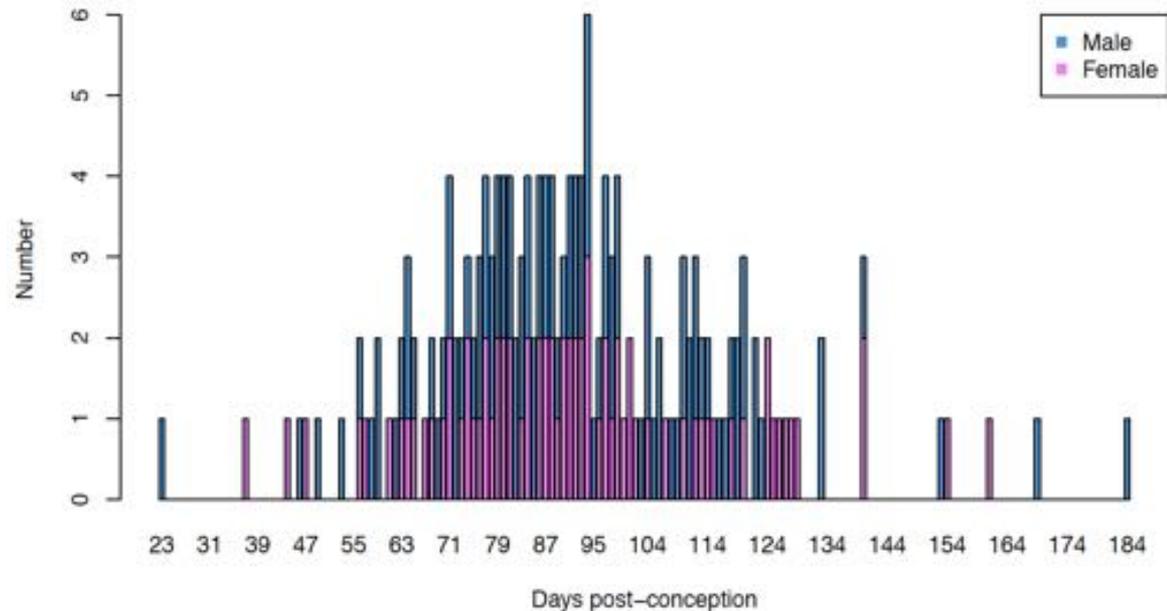
Research

## Methylomic trajectories across human fetal brain development

Helen Spiers,<sup>1</sup> Ellis Hannon,<sup>2</sup> Leonard C. Schalkwyk,<sup>3</sup> Rebecca Smith,<sup>1</sup> Chloe C.Y. Wong,<sup>1</sup> Michael C. O'Donovan,<sup>4</sup> Nicholas J. Bray,<sup>1</sup> and Jonathan Mill<sup>1,2</sup>

<sup>1</sup>Institute of Psychiatry, Psychology & Neuroscience, King's College London, London SE5 8AF, United Kingdom; <sup>2</sup>University of Exeter Medical School, University of Exeter, Exeter EX2 5DW, United Kingdom; <sup>3</sup>School of Biological Sciences, University of Essex, Colchester CO4 3SQ, United Kingdom; <sup>4</sup>MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University School of Medicine, Cardiff CF24 4HQ, United Kingdom

DNA methylation  
DNA hydroxymethylation  
RNA-seq (Nick Bray)  
Genetic variation  
Single-cell transcriptomics



179 human fetal brain samples profiled for DNA methylation

100 male, 79 females

23 to 184 days post-conception

DPC calculated by Carnegie staging and fetal foot length

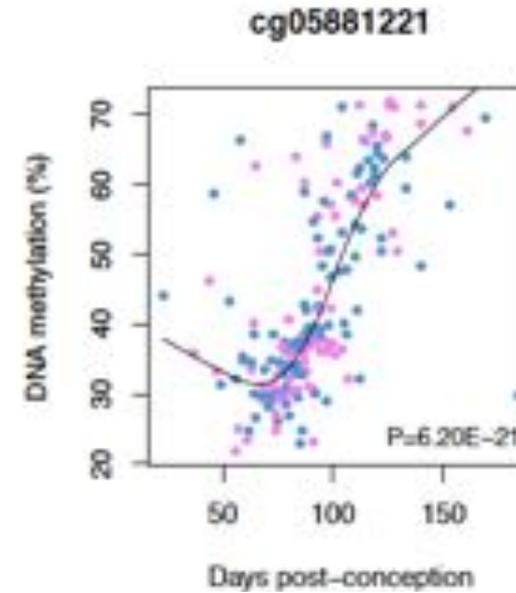
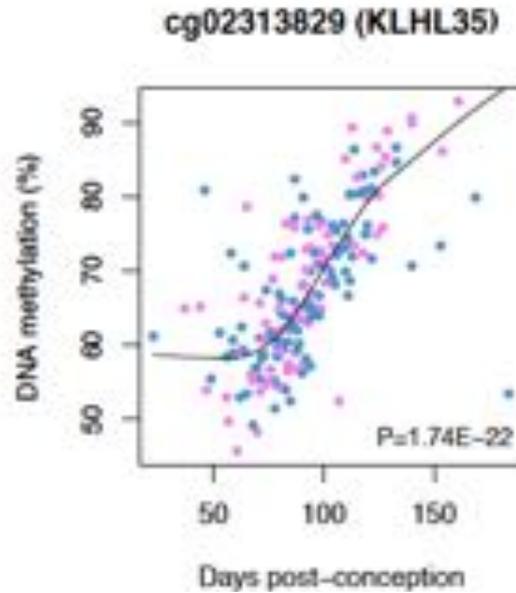
MRC Medical Research Council

HDBR

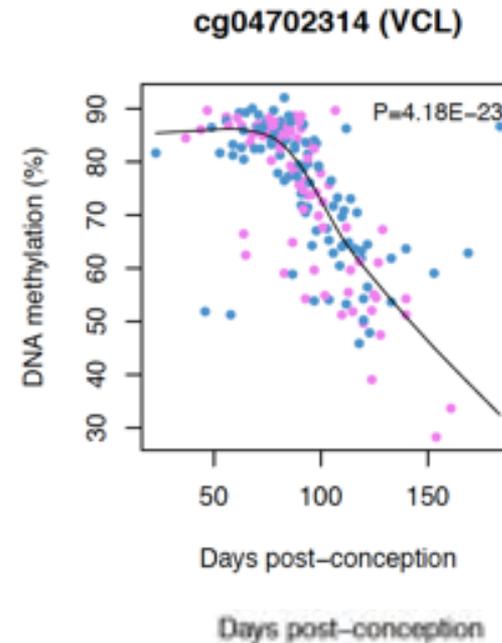
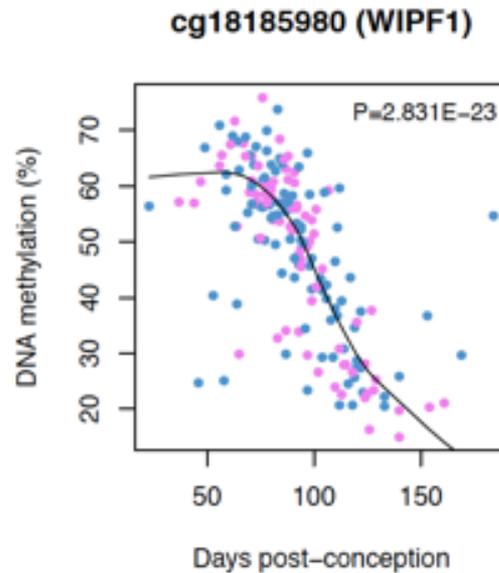
Human Developmental Biology Resource

>28,000 DMPs (annotated to >5,000 genes) significantly associated with fetal brain development

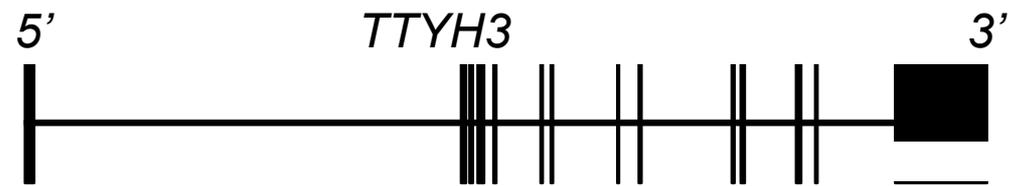
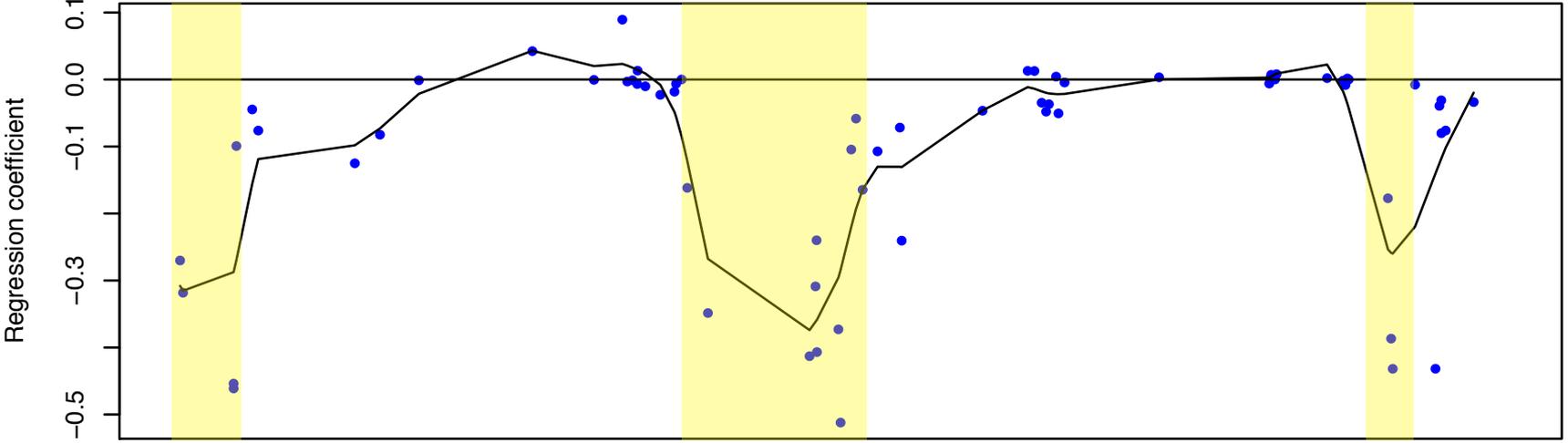
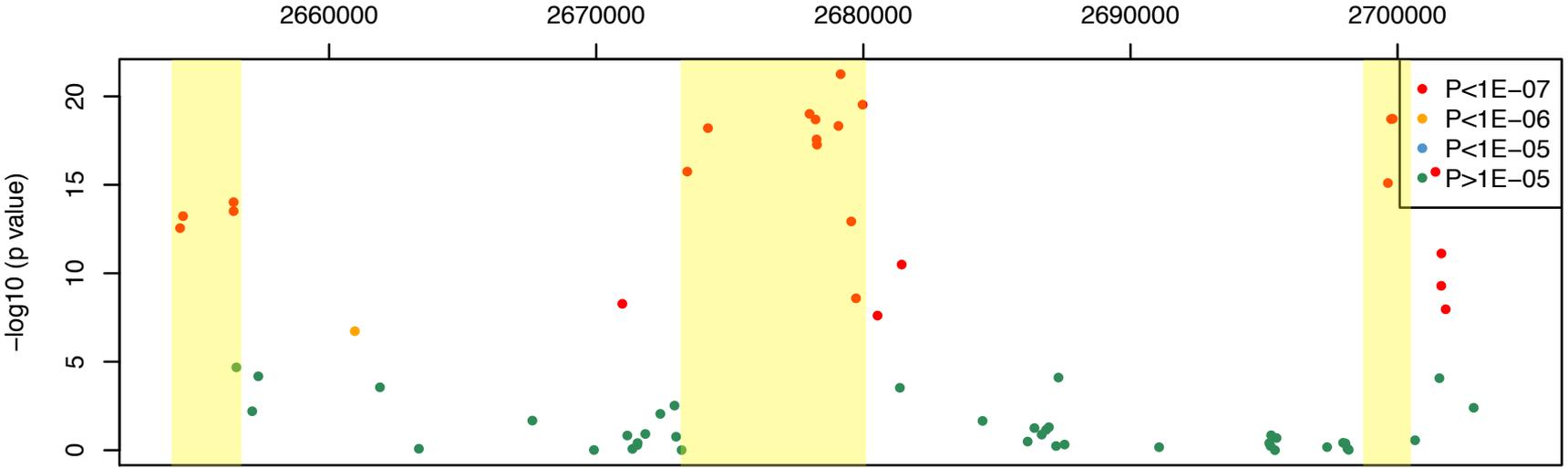
Hypermethylation



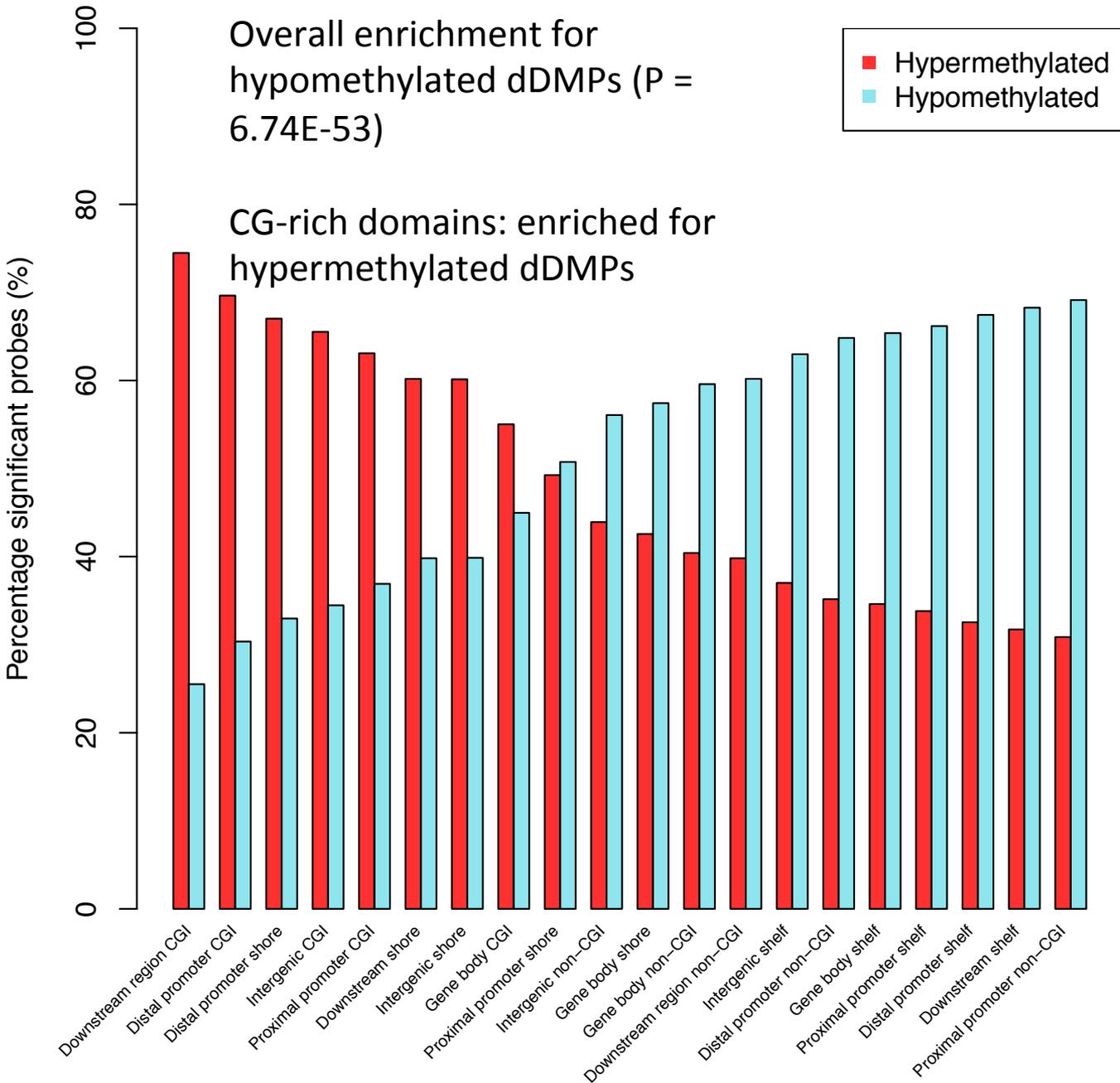
Hypomethylation



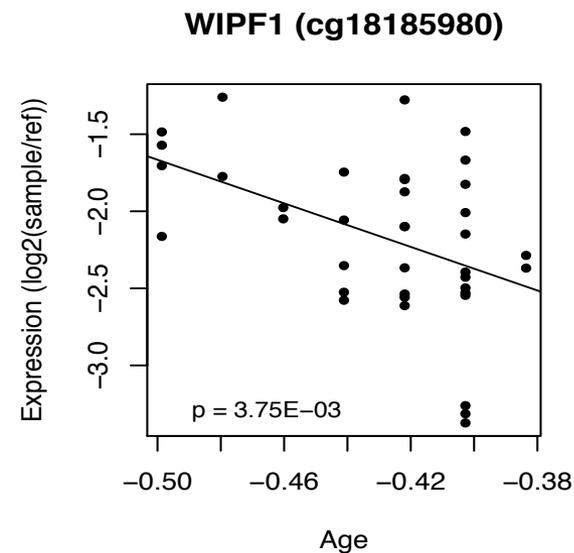
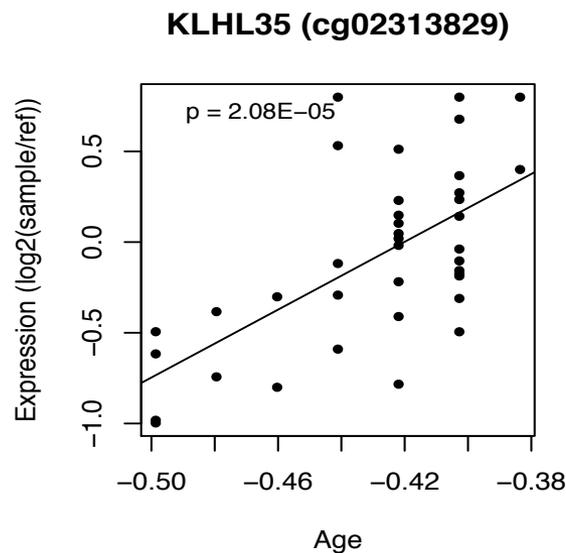
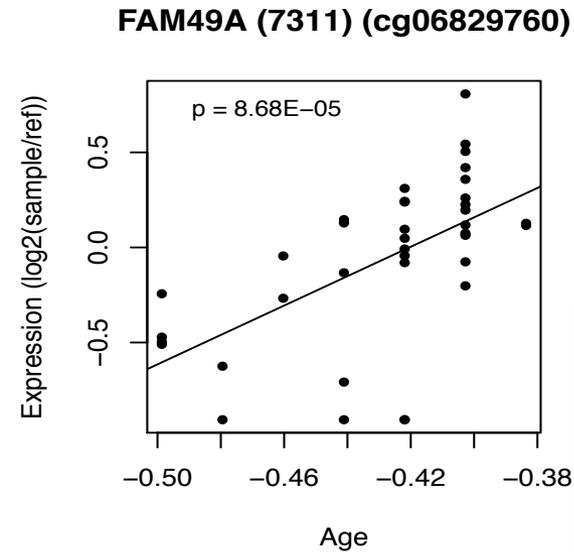
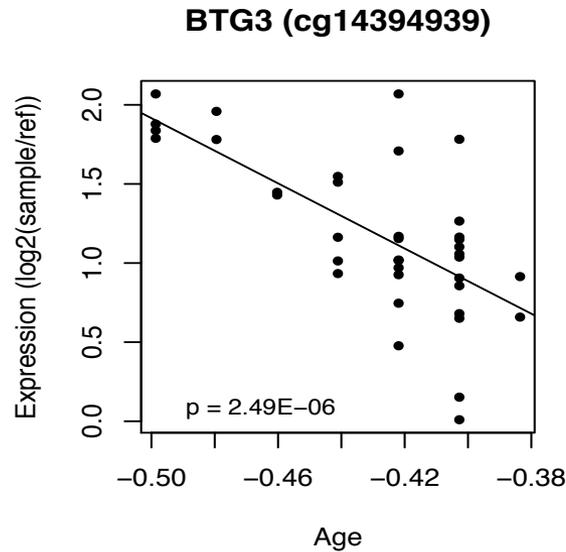
# Clustered regions of developmentally-coordinated DNA methylation in the human fetal brain.



# The distribution and direction of fetal brain dDMPs is not equal across genomic regions...



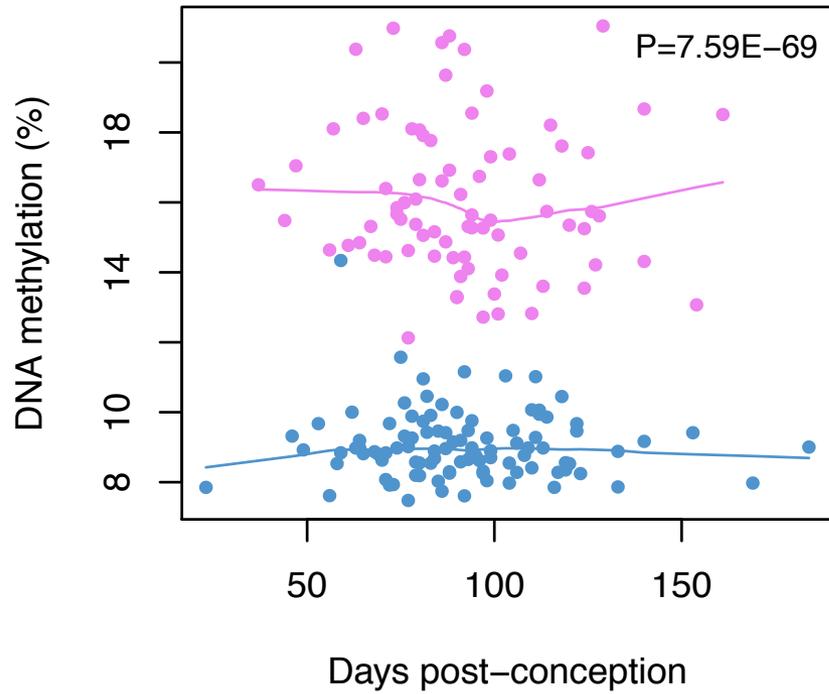
# Correlation with gene expression data from Brain Cloud resource (<http://braincloud.jhmi.edu>)



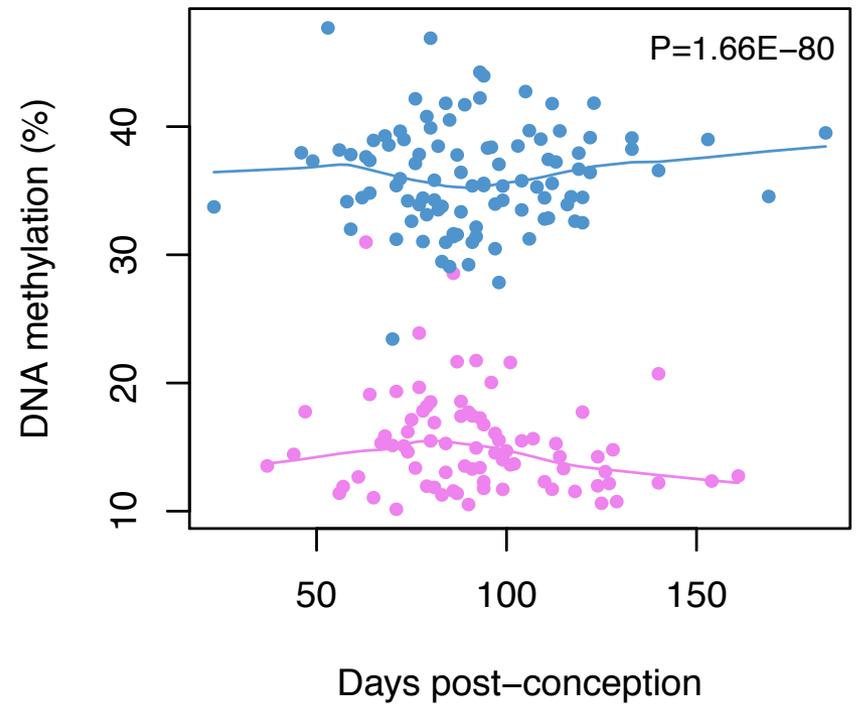
~30% of top-ranked dDMPs annotated to a gene that is dynamically-expressed during brain development

There are autosomal sex differences ...

**cg03691818 (KRT77)**

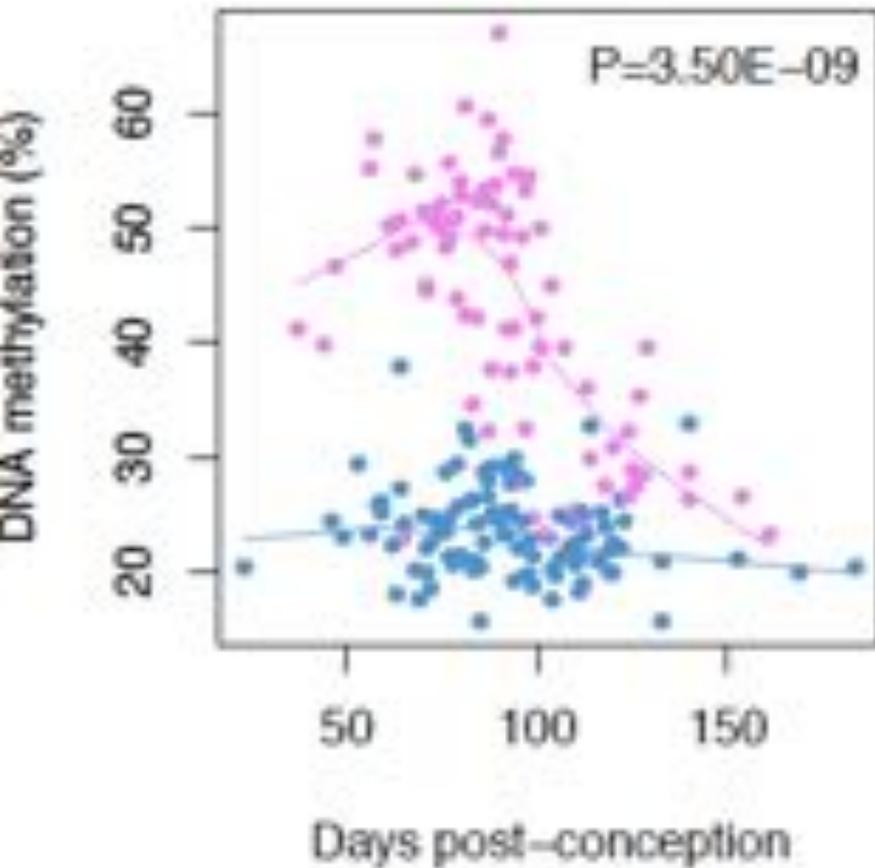


**cg12691488**

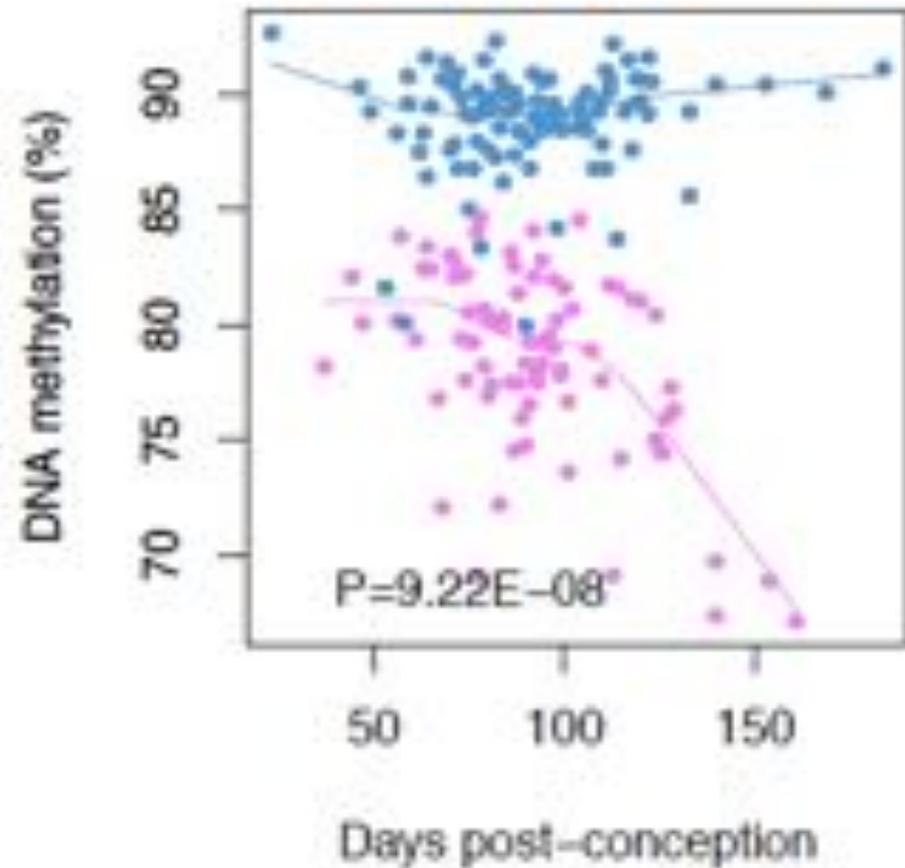


There are autosomal sex differences and distinct sex-specific developmental trajectories in the human fetal brain methylome

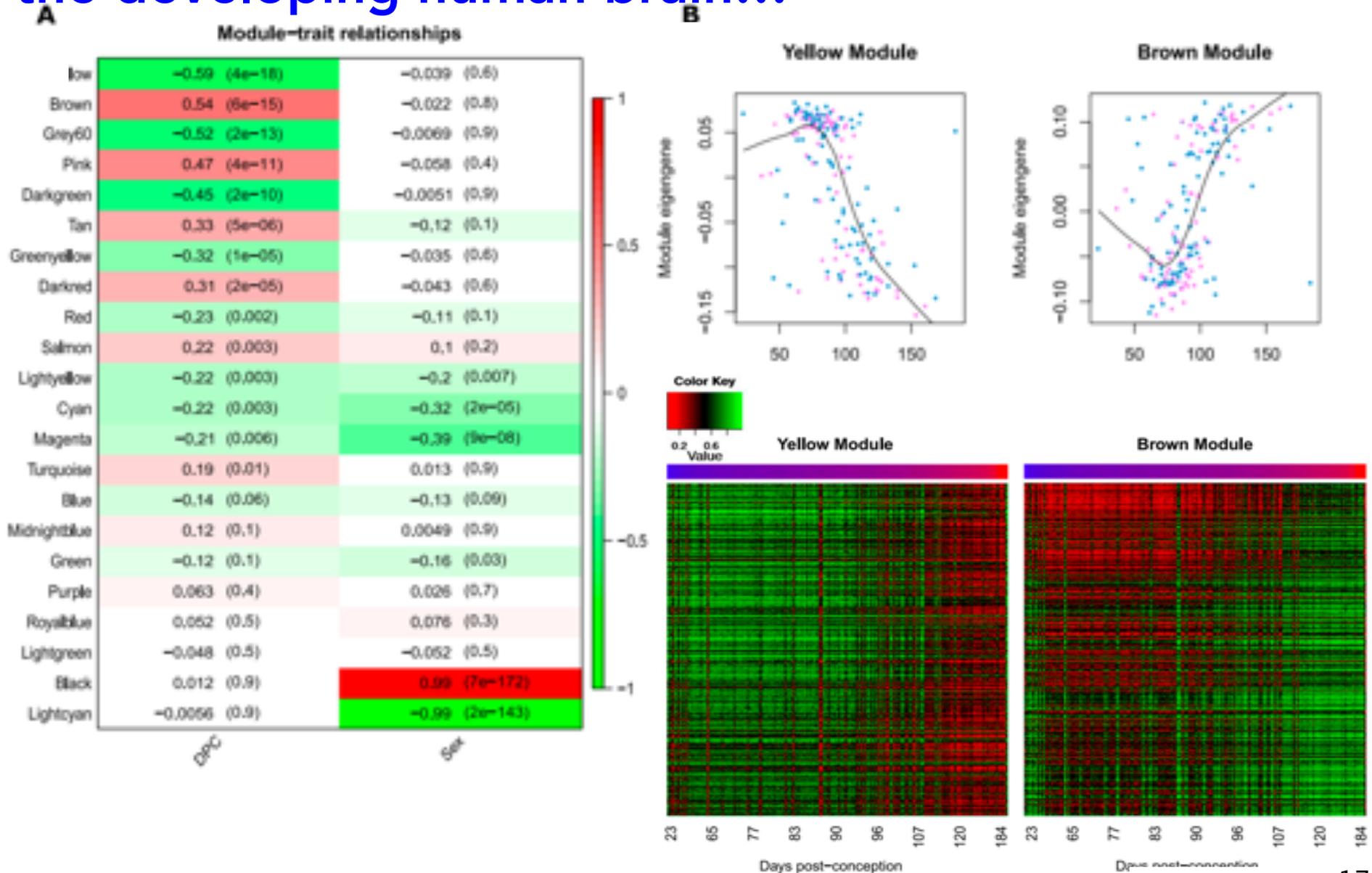
cg09167861 (FHL1)



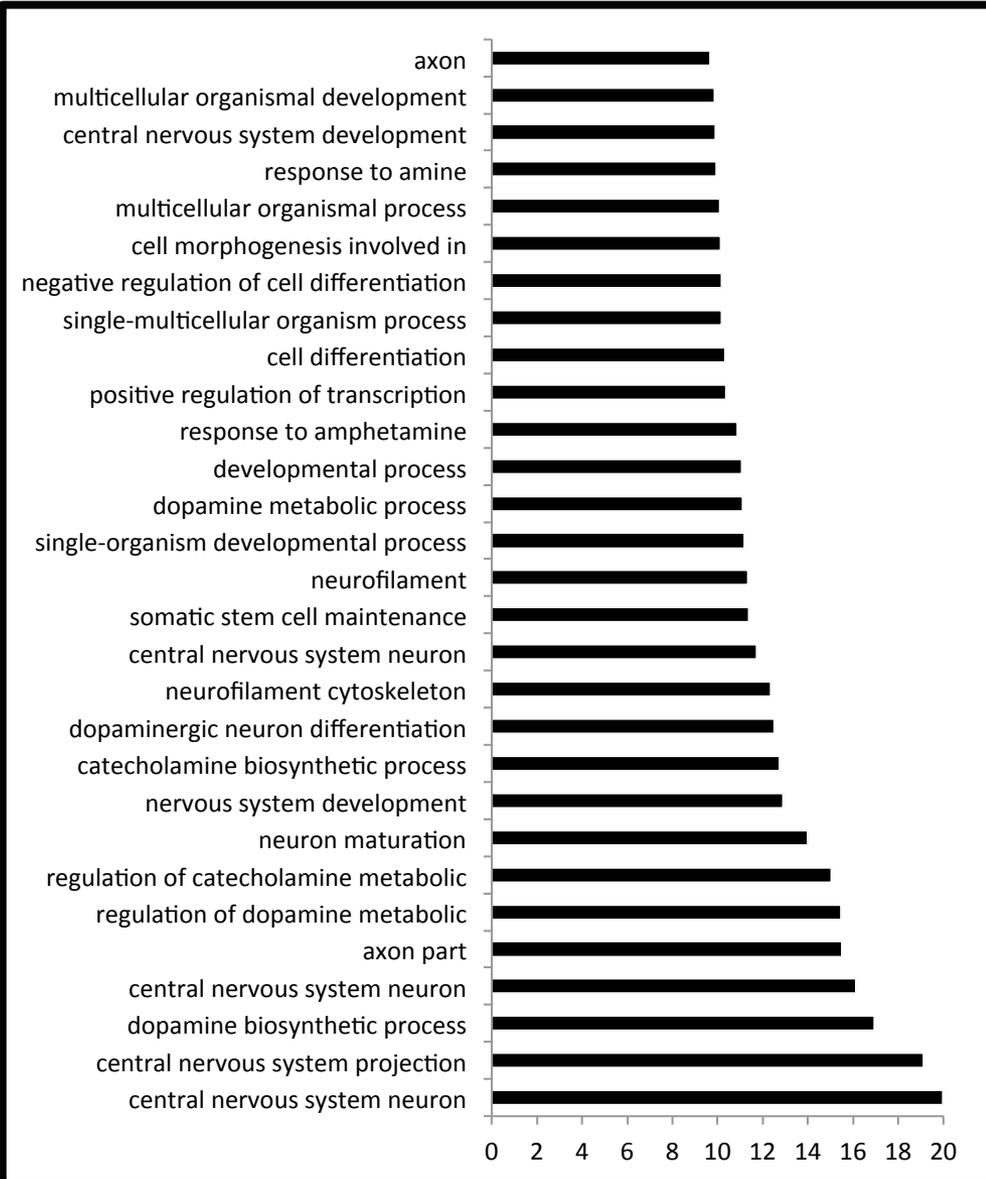
cg00718858



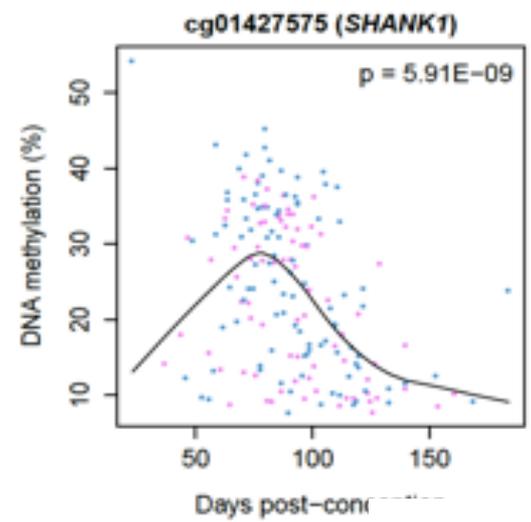
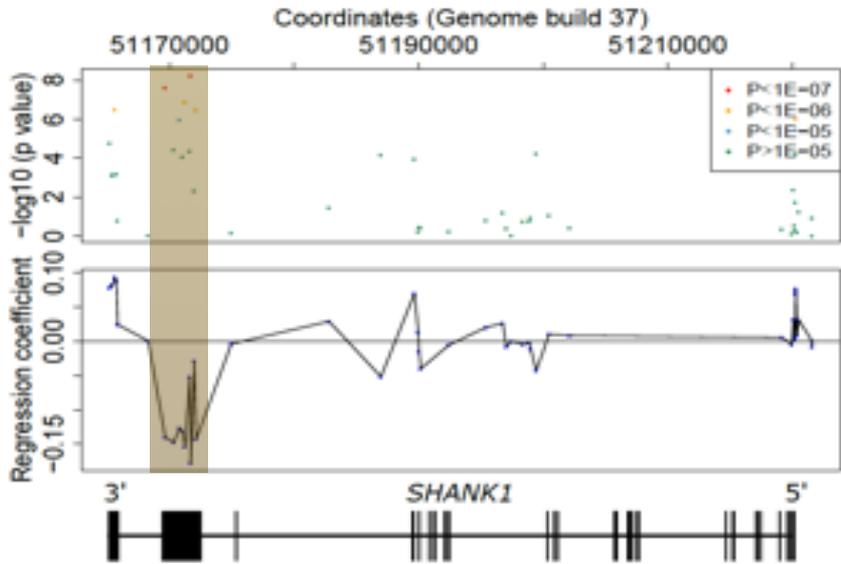
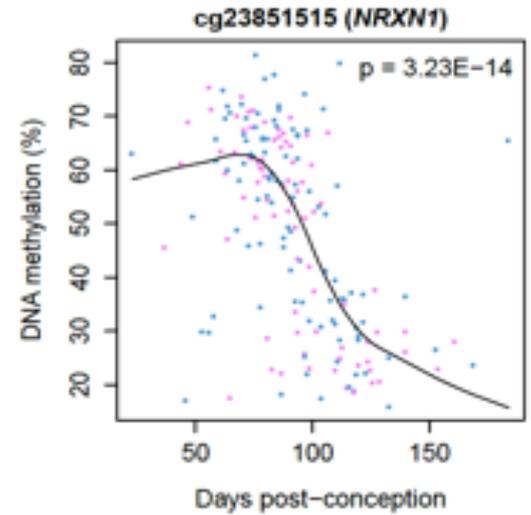
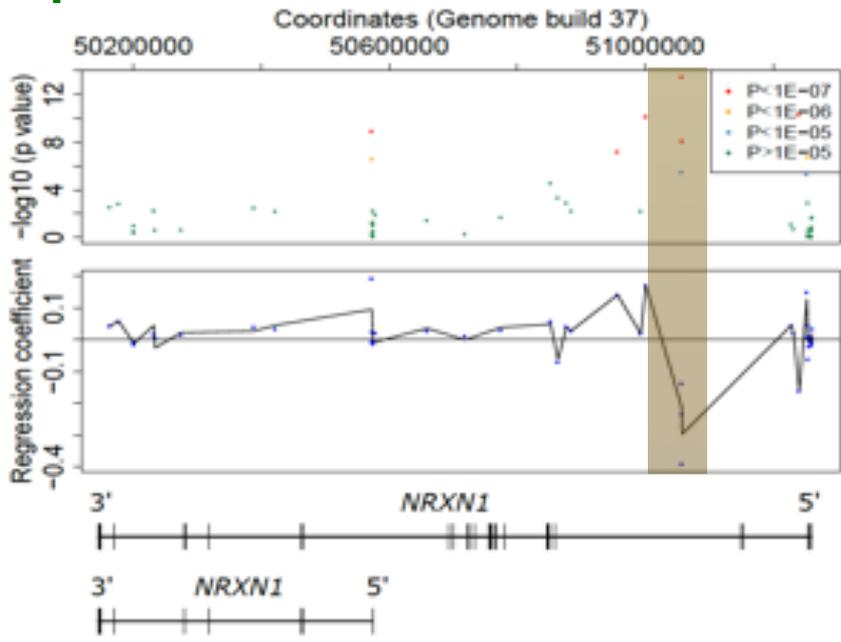
# There are distinct modules of co-methylated loci in the developing human brain...



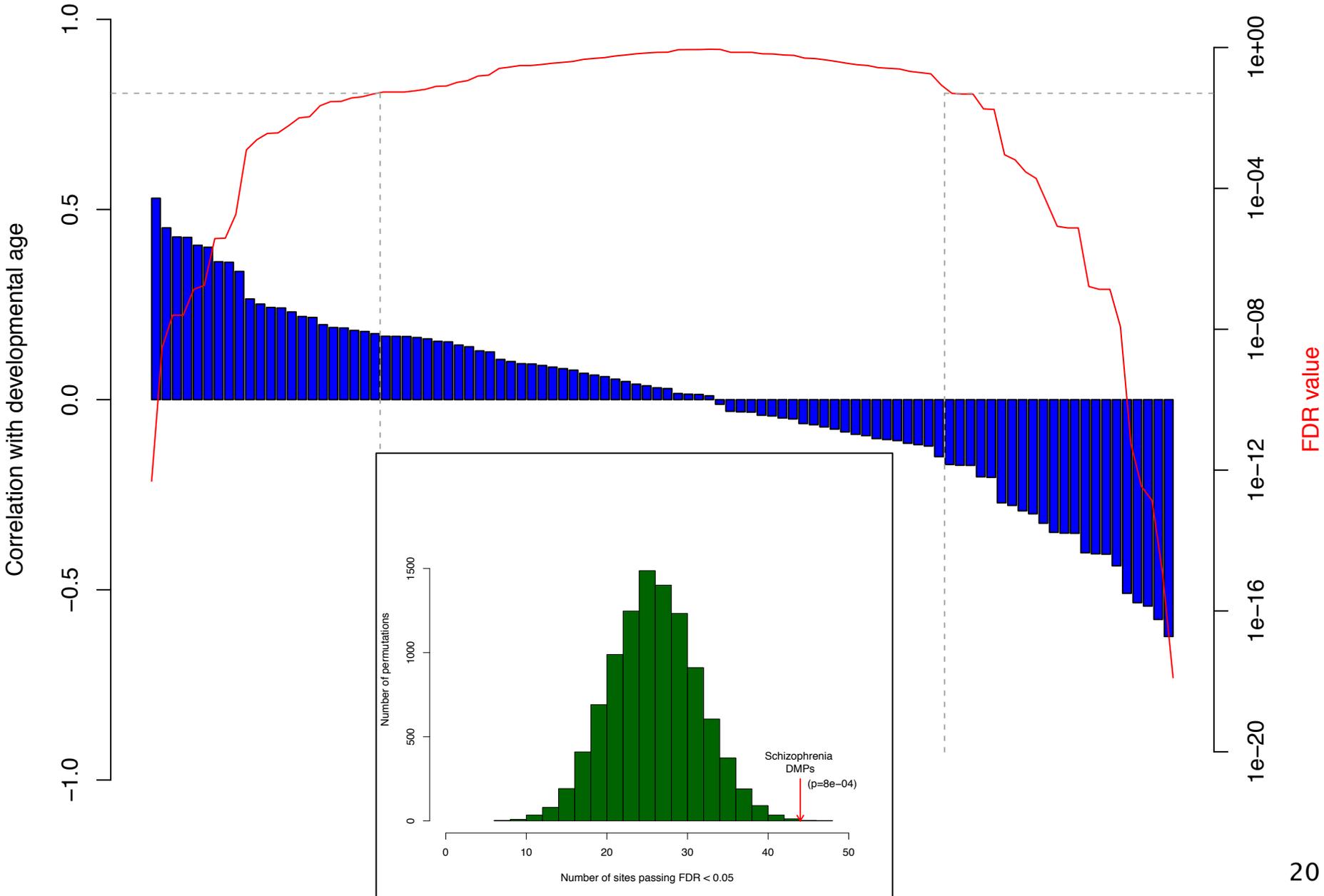
# ...which are highly enriched for neurodevelopmental processes.

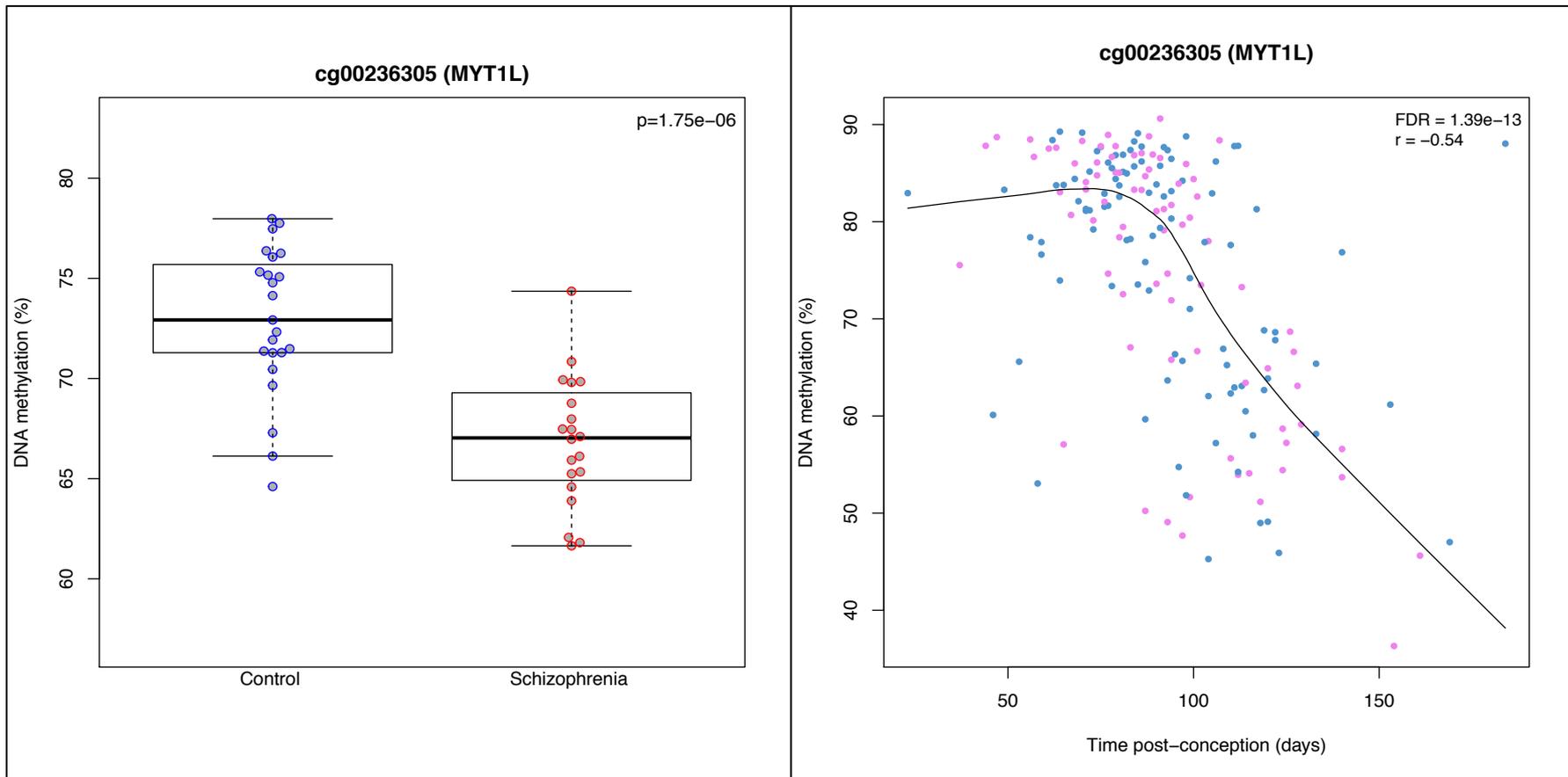


# Brain development DMPs are enriched in genes linked to neurodevelopmental disorders



# Schizophrenia-associated DNA methylation differences in PFC are significantly enriched for neurodevelopmentally-dynamic sites.

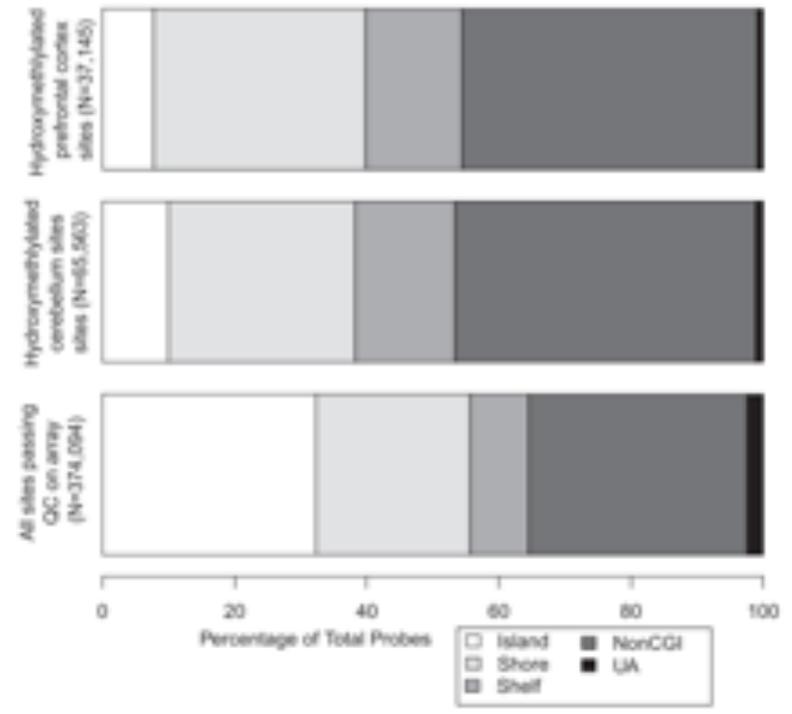
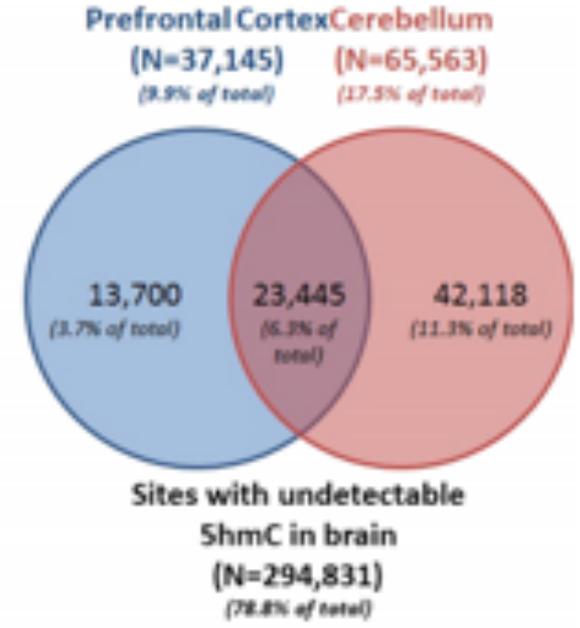




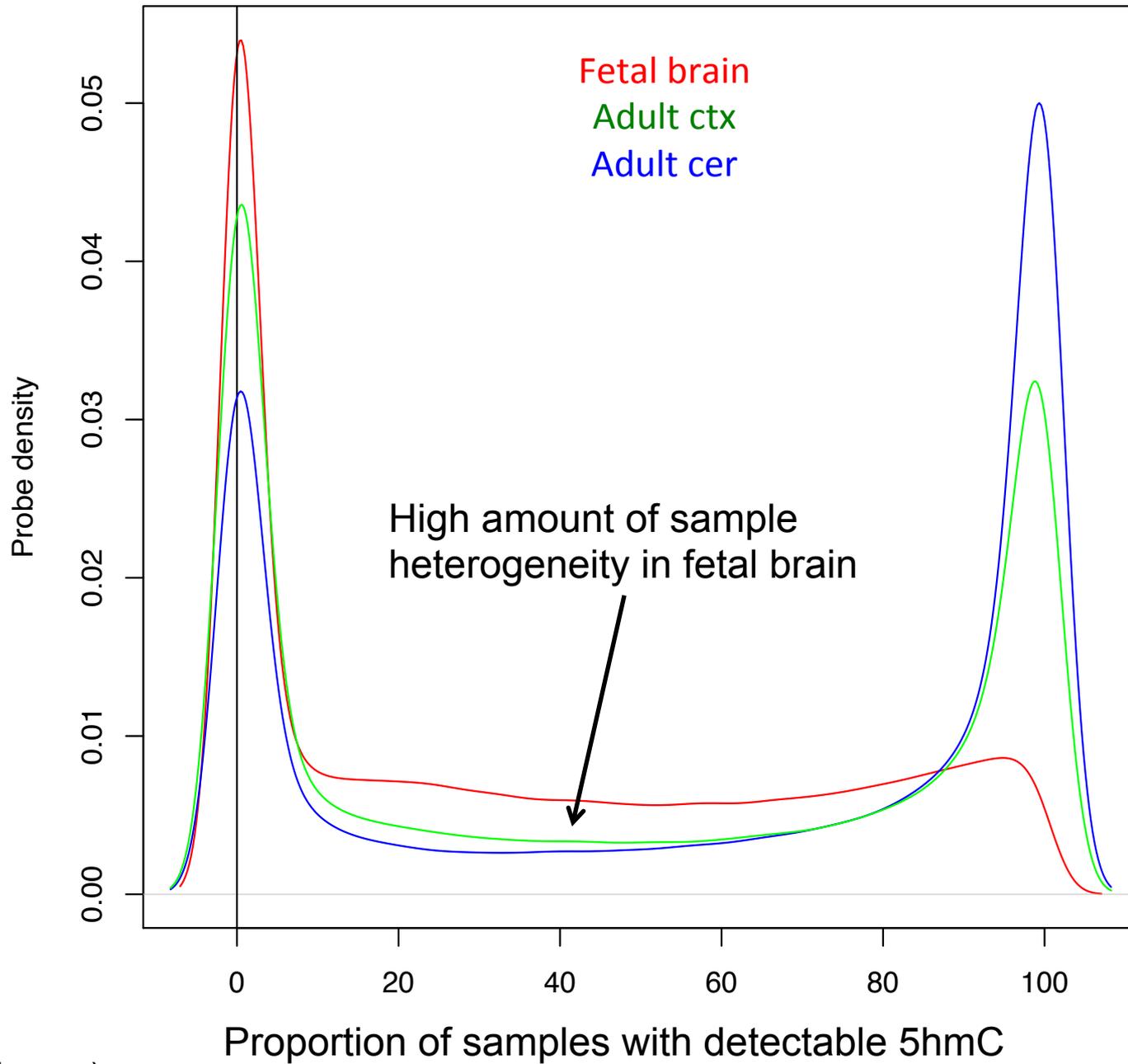


## Variation in 5-hydroxymethylcytosine across human cortex and cerebellum

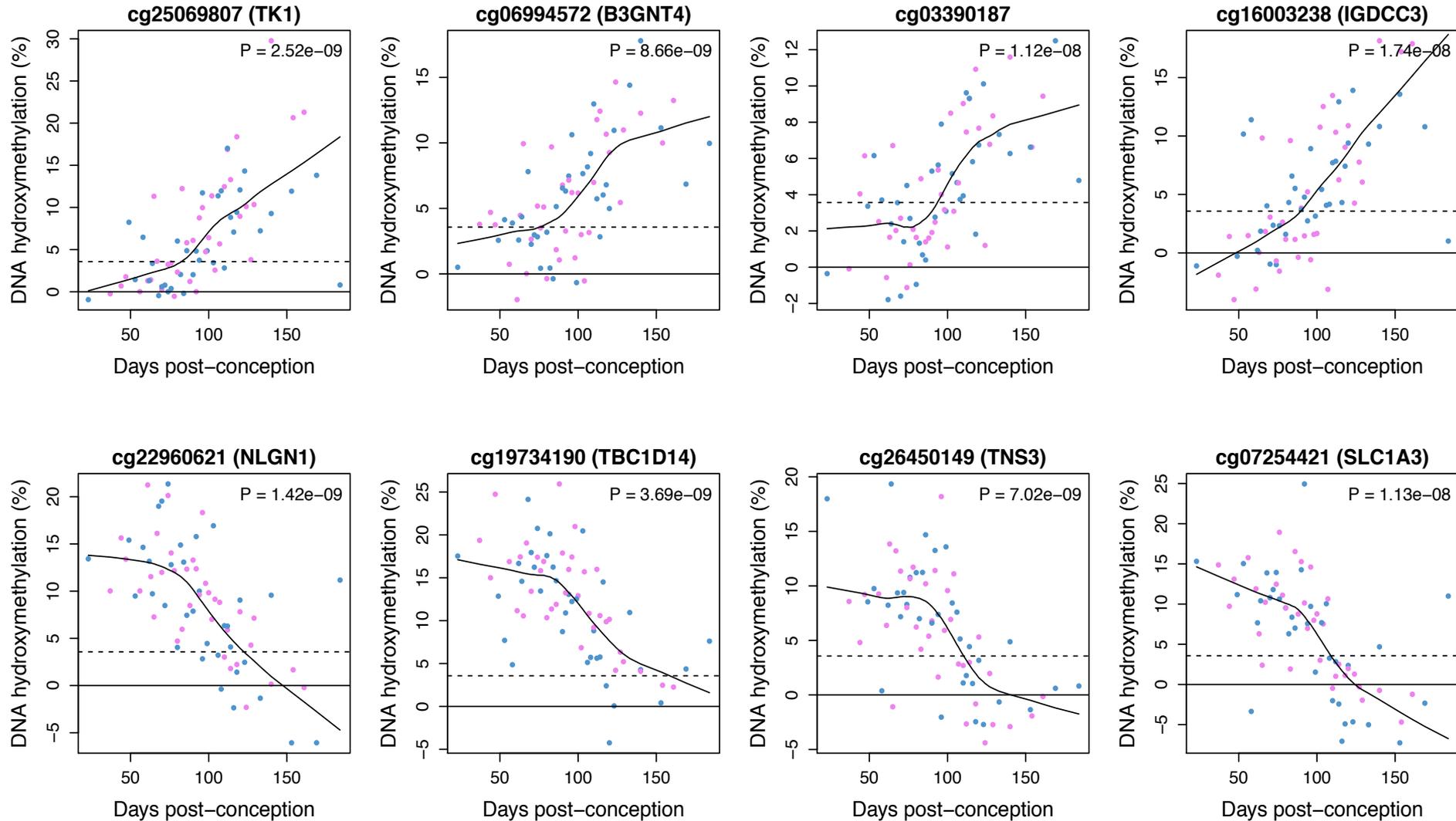
Lunnon et al.



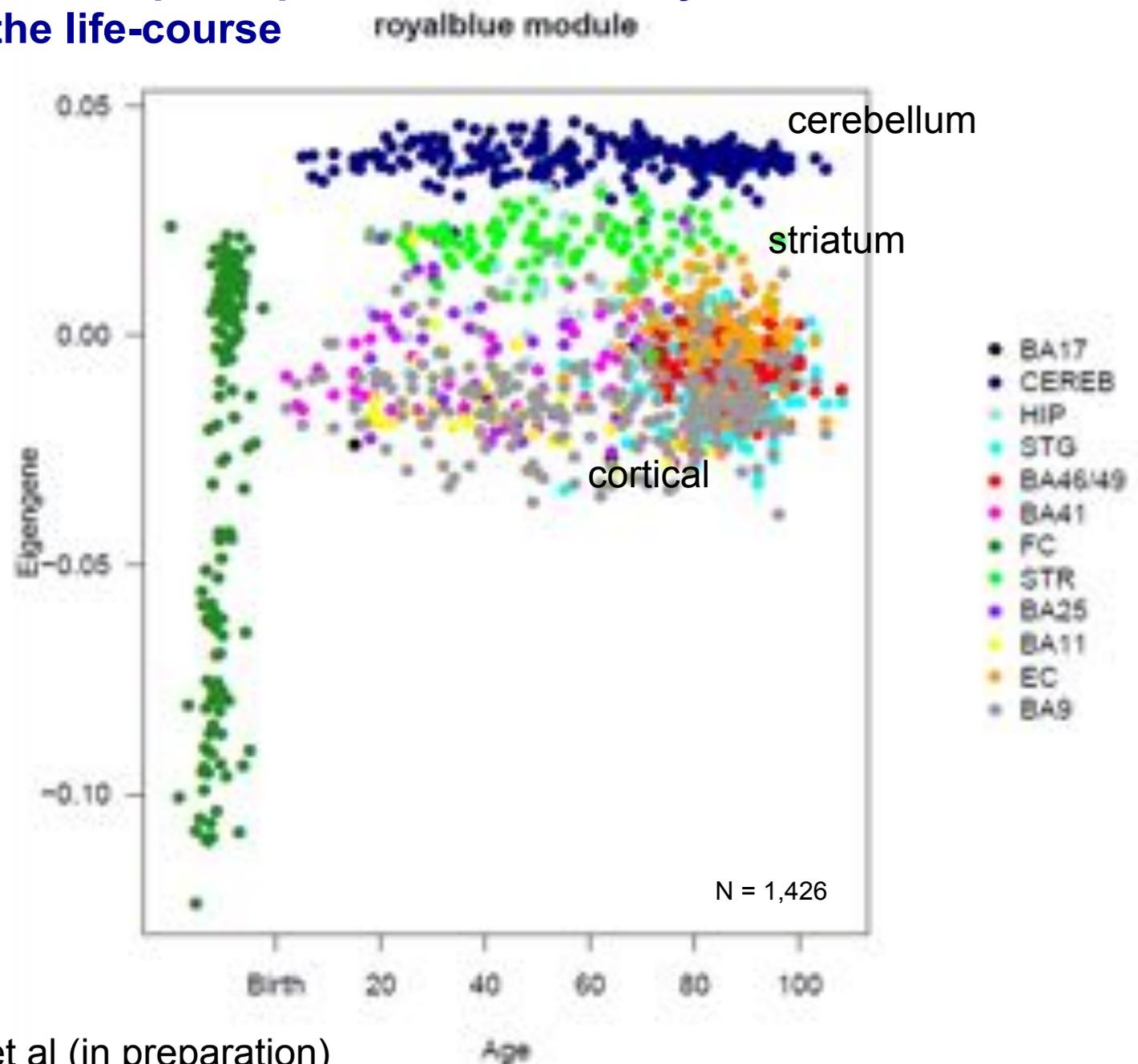
5hmC enriched in cerebellum 5hmC generally not in promoter CGIs



# DNA hydroxymethylation (5hmC) is highly dynamic across brain development at individual loci



# Temporal and spatial patterns of co-methylation in the human brain across the life-course



# Genetic epidemiology

- 1 body, 1 genome: all you need is a blood sample
- 1 life, 1 genome: you are born with the genome you die with
- Any lifestyle, 1 genome: it doesn't matter what you're exposed to
- Any disease, 1 genome: no reverse causation
- A well annotated reference genome and catalogue of polymorphic variants
- Methods that do as they say on the box and give results that are easy to interpret

# EpiGenetic epidemiology

- 1 body, 1 genome, all you need is a blood sample
- 1 life, 1 genome: you are born with the genome you die with
- Any life, 1 genome doesn't matter what you're exposed to
- Any disease, 1 genome, no reverse causation
- A well annotated reference genome and catalogue of polymorphisms
- Methods that take the box and give results that are easy to interpret

To what extent can easily-accessible peripheral tissues/cells (e.g. whole blood, saliva, buccal) be used as a proxy for inaccessible tissues/cells (i.e. brain)?

RESEARCH PAPER

Epigenetics 10:11, 1024–1032; November 2015; Published with license by Taylor & Francis Group, LLC

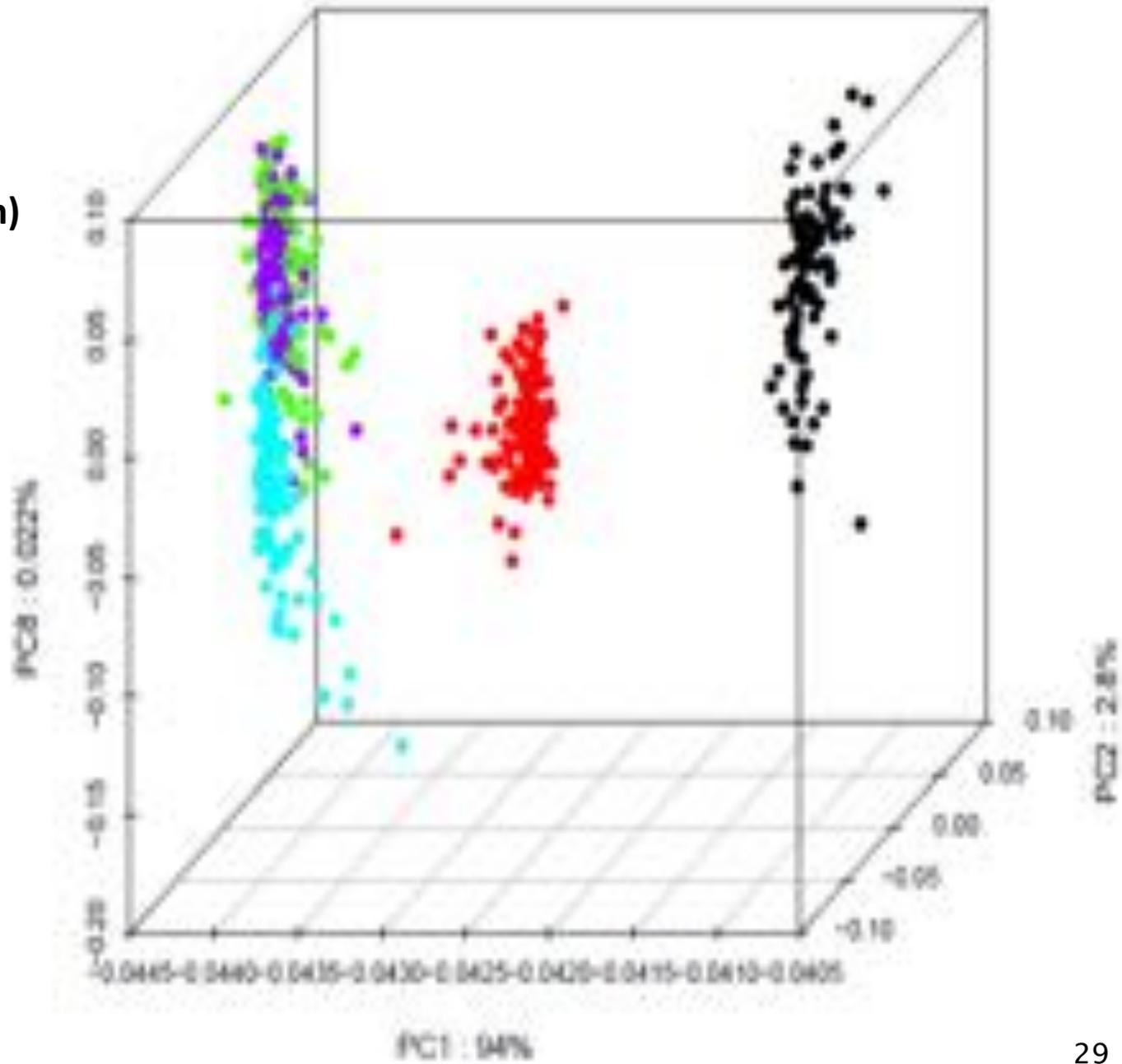
## Interindividual methylomic variation across blood, cortex, and cerebellum: implications for epigenetic studies of neurological and neuropsychiatric phenotypes

Ellis Hannon<sup>1</sup>, Katie Lunnon<sup>1</sup>, Leonard Schalkwyk<sup>2</sup>, and Jonathan Mill<sup>1,3,\*</sup>

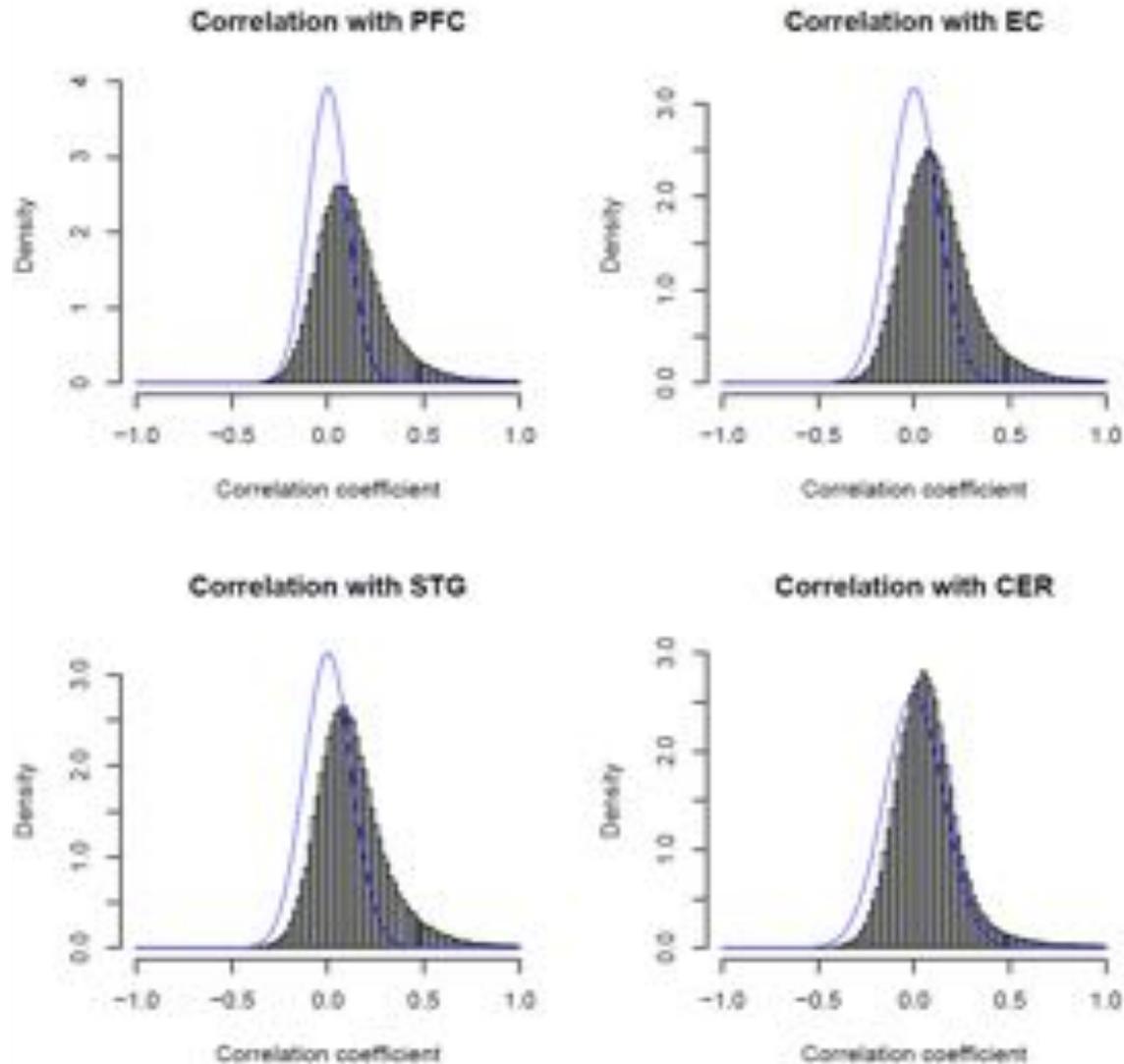
Profiled five matched tissues from ~100 individuals  
Illumina 450K array

- Whole Blood (pre-mortem)
- Cerebellum
- Superior Temporal Gyrus
- Entorhinal Cortex
- Prefrontal Cortex

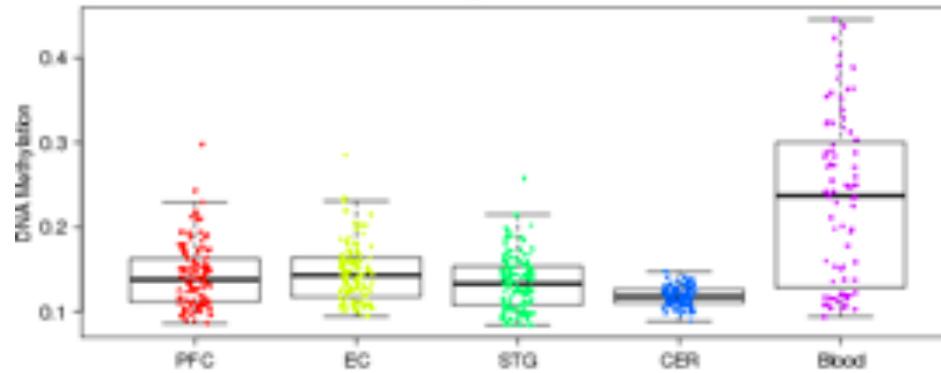
Dramatically more variation between tissues within an individual than between individuals within a tissue



# The important question for epigenetic epidemiology / biomarker research: is inter-individual variation correlated across tissues?

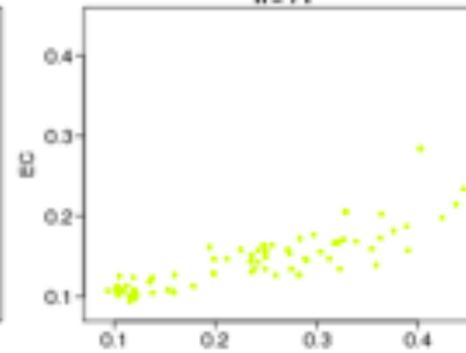
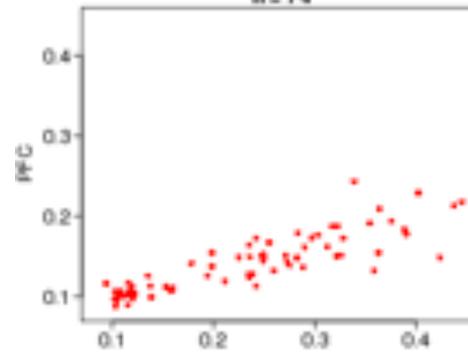


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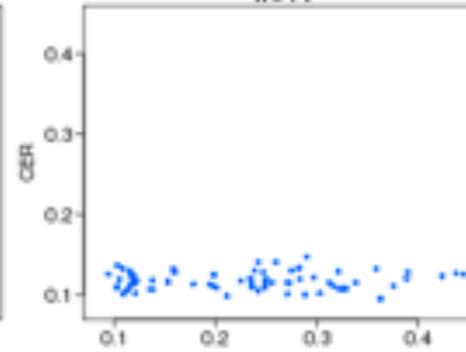
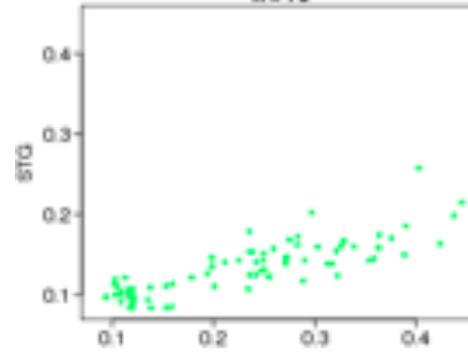
$r = 0.867$   $p$  value = 0  
 $n = 74$

$r = 0.855$   $p$  value = 0  
 $n = 71$



$r = 0.832$   $p$  value = 0  
 $n = 75$

$r = 0.0595$   $p$  value = 0.622  
 $n = 71$



<http://epigenetics.iop.kcl.ac.uk/bloodbrain/>

# From promises to practical strategies in epigenetic epidemiology

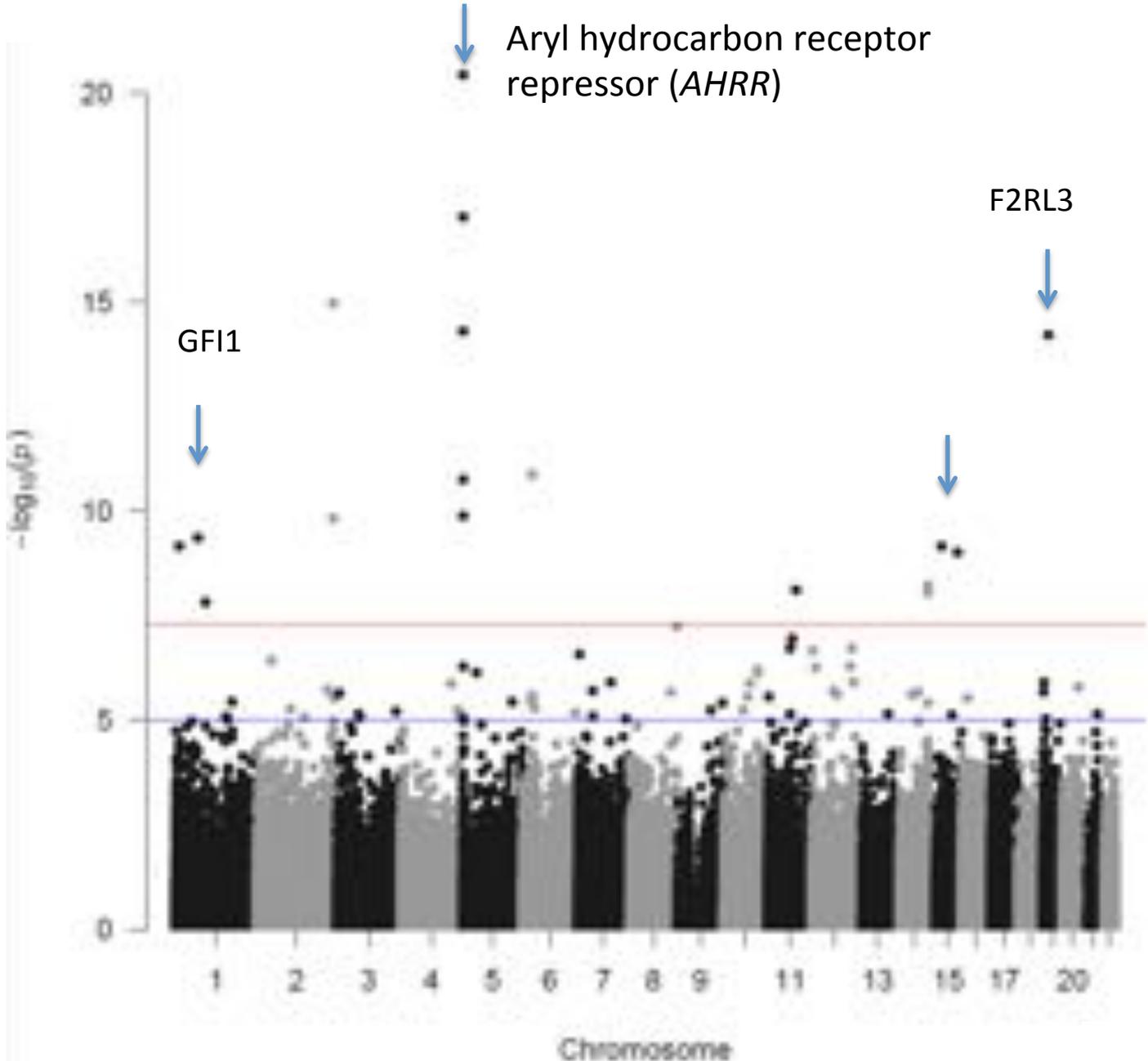
Jonathan Mill and Bastiaan T. Heijmans



- Small numbers
- Inappropriate tissues/cells
- Candidate gene focused
- Sub-optimal study designs
- Hype and over-interpretation**



# Blood SZ EWAS – Phase 1 (UCL)

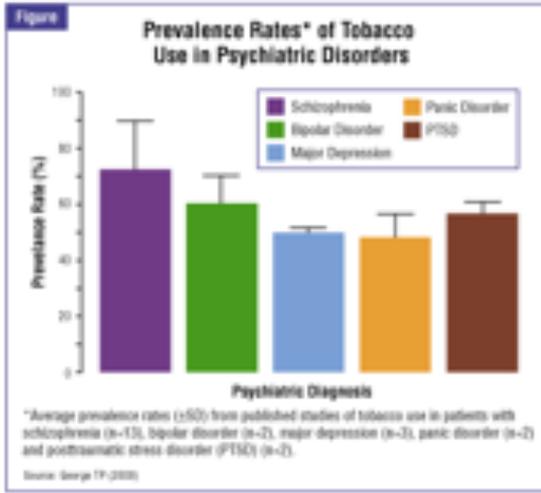
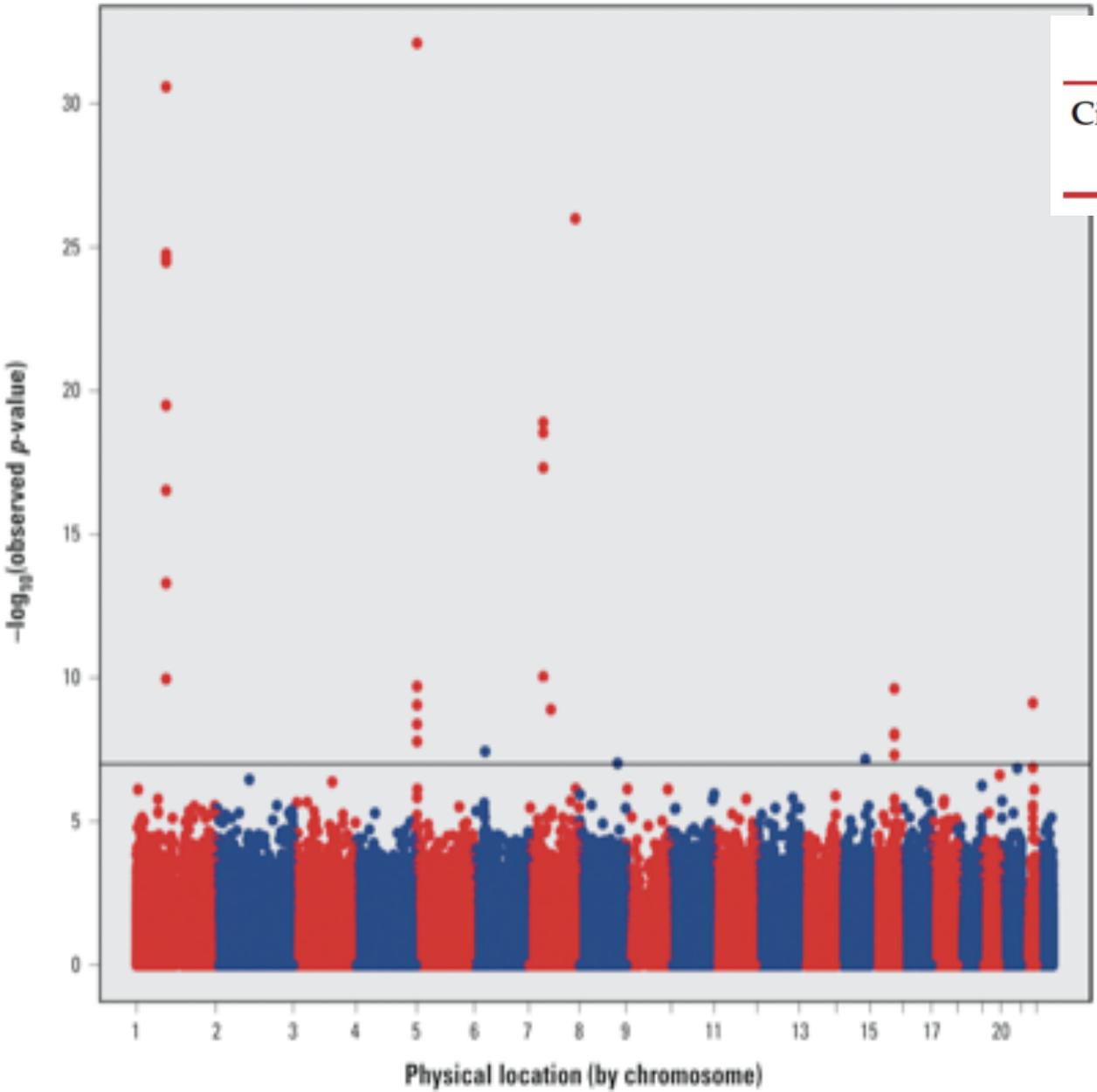


# Smoking EWAS

Advances in Psychiatric Treatment (2000), vol. 6, pp. 327-331

## Cigarette smoking and schizophrenia

Ciara Kelly & Robin McCreadie



US population: 25-30%  
Schizophrenics: 70-90%

# Can we infer smoking status from DNA methylation data?

PLoS ONE | Clinical Epigenetics | Research Article

**RESEARCH** Open Access

## Differences in smoking associated DNA methylation patterns in South Asians and Europeans

Harshil K. Shah<sup>1,2</sup>, Thomas Willer<sup>1,2</sup>, Manjiv J. Mehta<sup>1,2</sup>, Koen Ho<sup>1,2</sup>, Ananya Choudhury<sup>1,2</sup>, Tom M. Frayling<sup>2</sup>, George Davey Smith<sup>1,2</sup>, Alan D. Hughes<sup>1,2</sup>, Ash Chaturvedi<sup>1,2</sup> and Corina E. Sillito<sup>1,2</sup>

**CLINICAL EPIGENETICS**

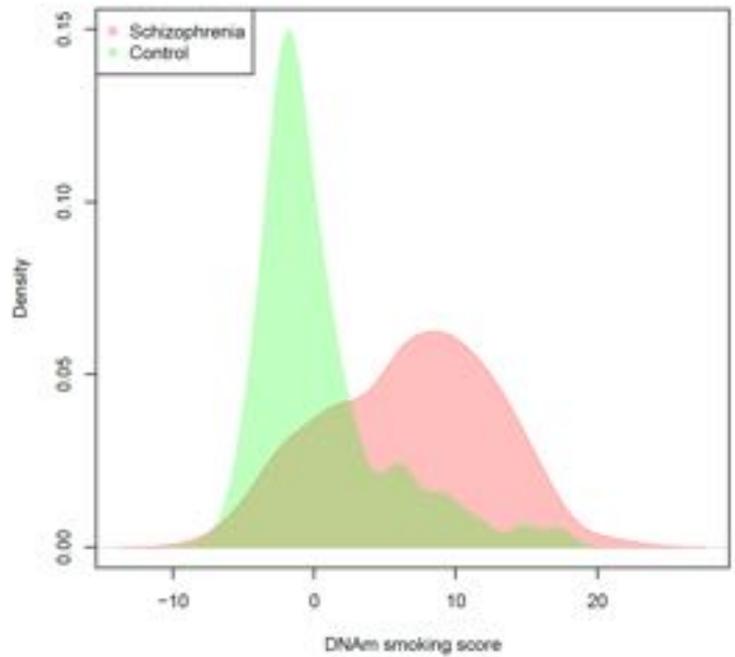
PLoS ONE | Research Article

## Tobacco Smoking Leads to Extensive Genome-Wide Changes in DNA Methylation

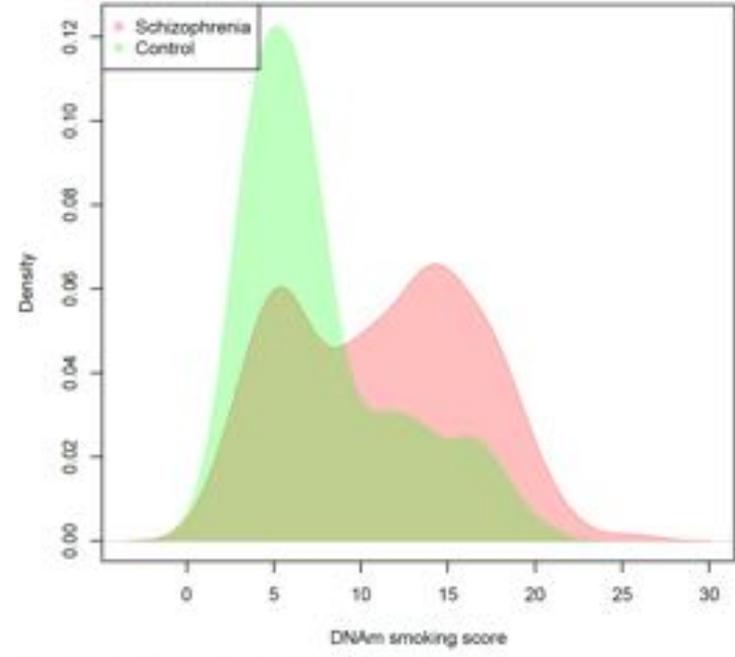
Seija Zillinger<sup>1</sup>, Brigitta Kühnel<sup>2</sup>, Norman Klopp<sup>1,3</sup>, Hansjörg Baurisch<sup>1,4</sup>, Anja Klumachenko<sup>1</sup>, Christian Gieger<sup>5</sup>, Stephan Weidinger<sup>6</sup>, Eva Laska<sup>1</sup>, Jerry Adami<sup>7,8</sup>, Annette Peters<sup>1,9</sup>, Konstantin Strauch<sup>1,10</sup>, Melanie Waldenberger<sup>1,11</sup>, Thomas Illig<sup>1,12</sup>

**Abstract:** ...

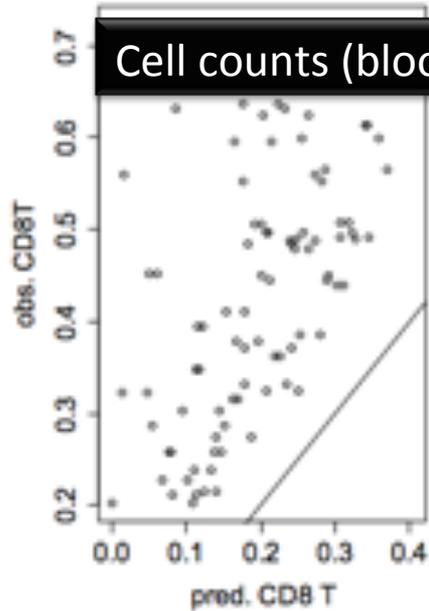
Phase 1



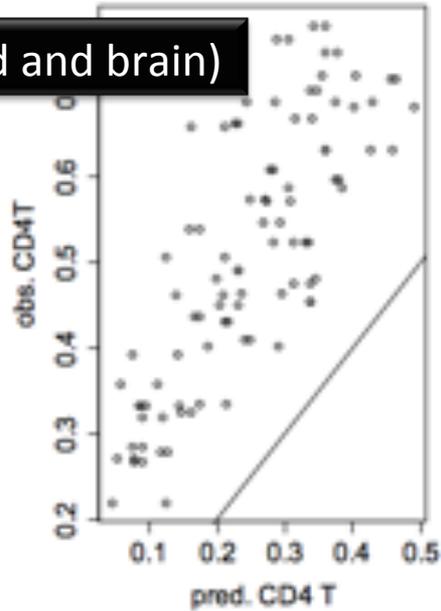
Phase 2



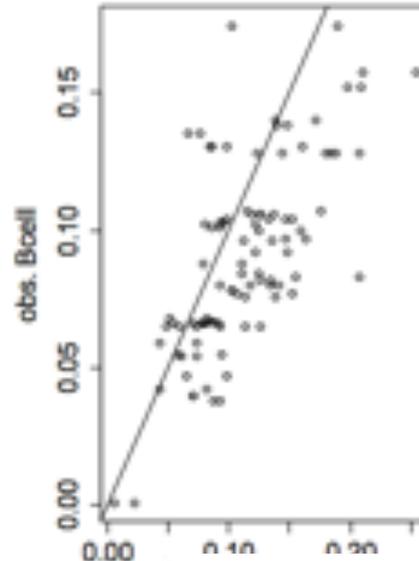
A bicor=0.63, p=6.1e-12



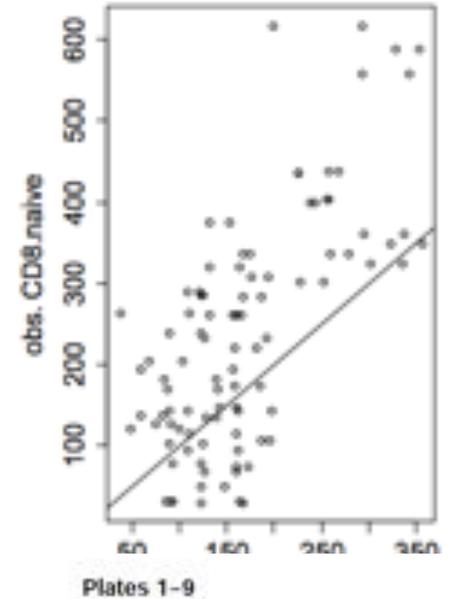
B bicor=0.77, p=4.8e-20



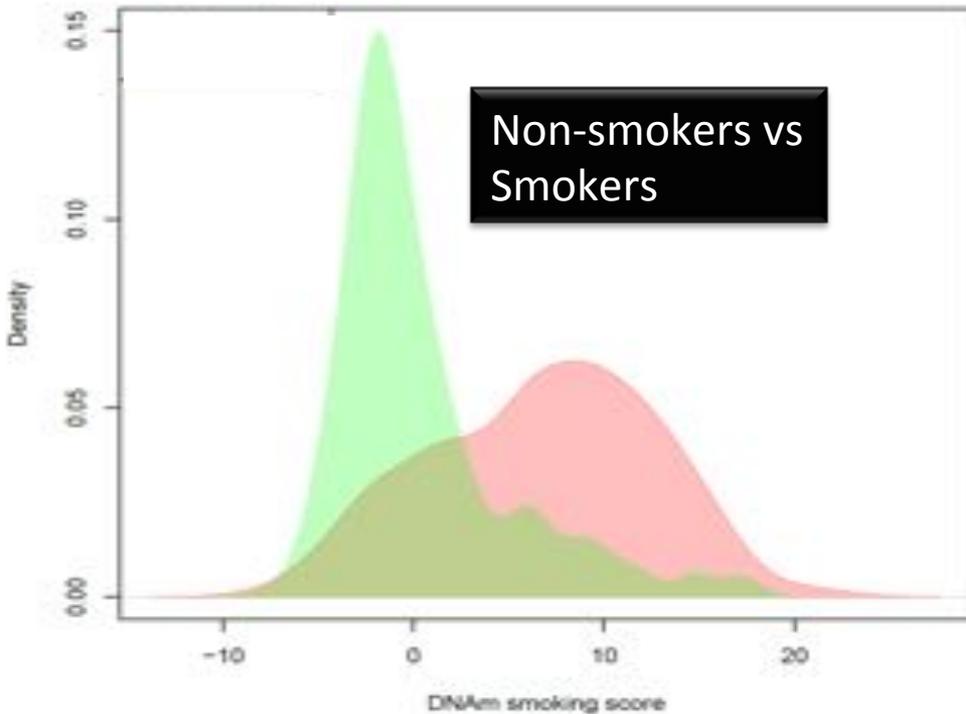
C bicor=0.67, p=8.3e-14



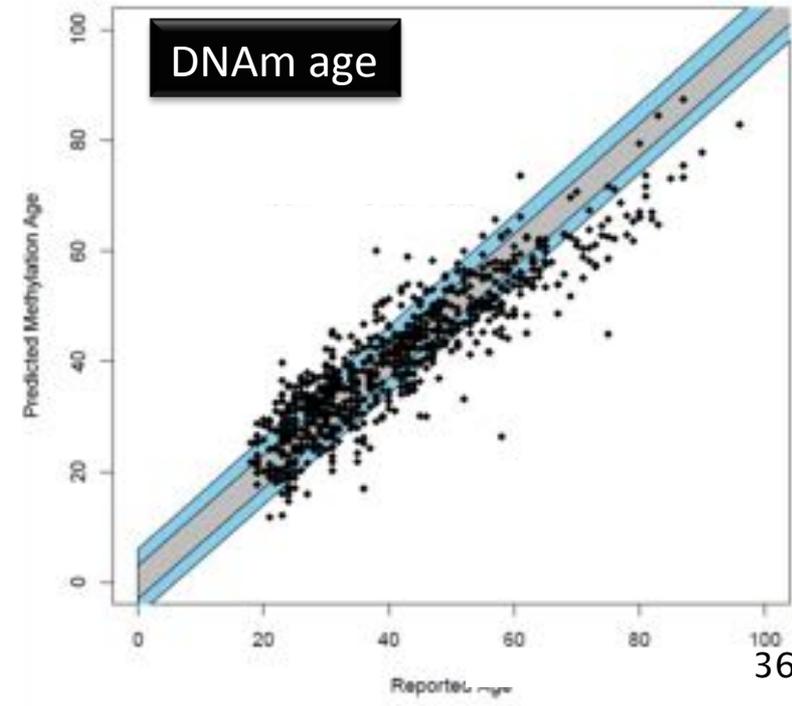
D bicor=0.68, p=2.6e-14



Non-smokers vs Smokers

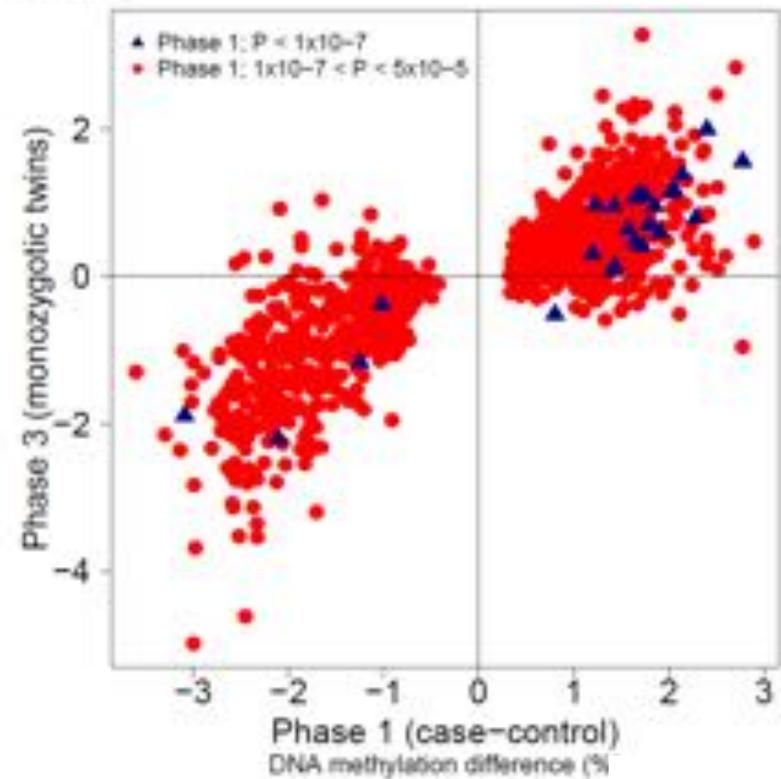
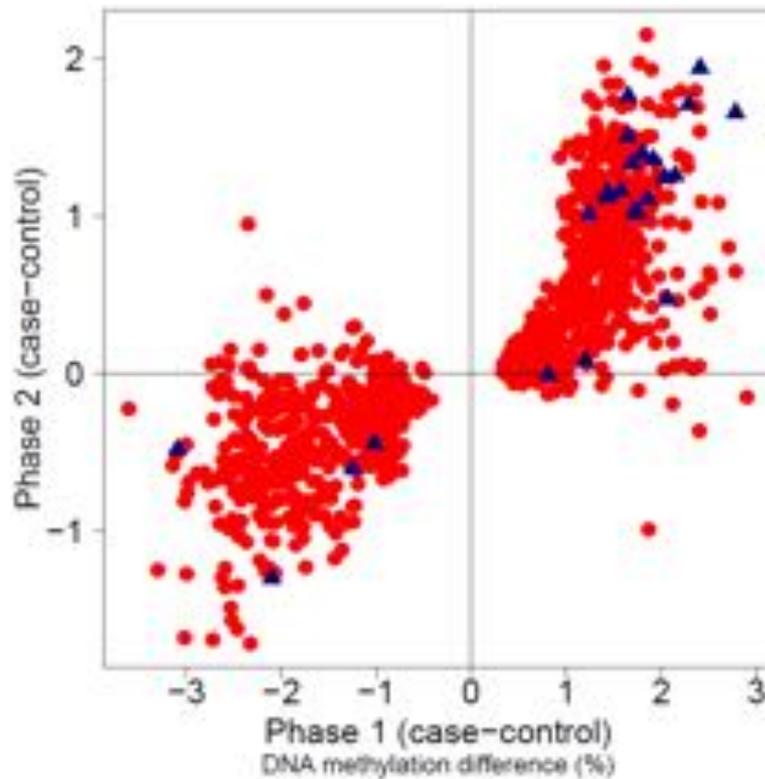
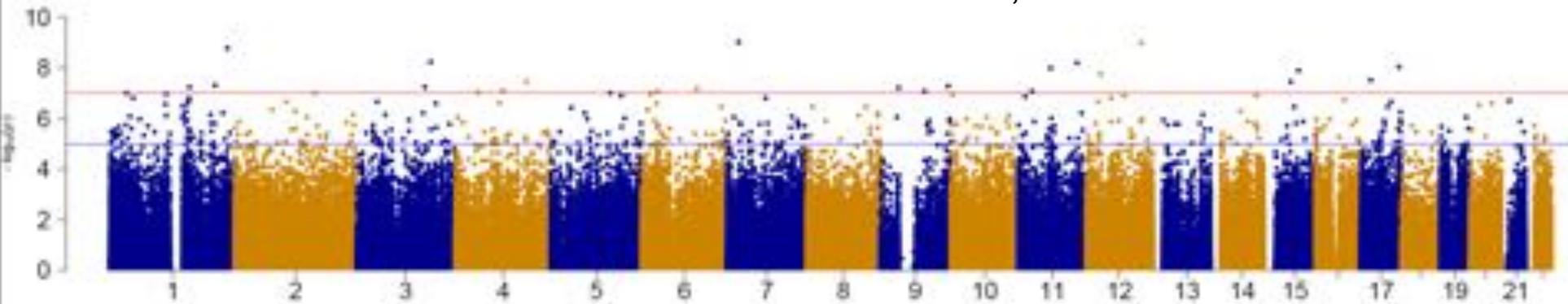


DNAm age

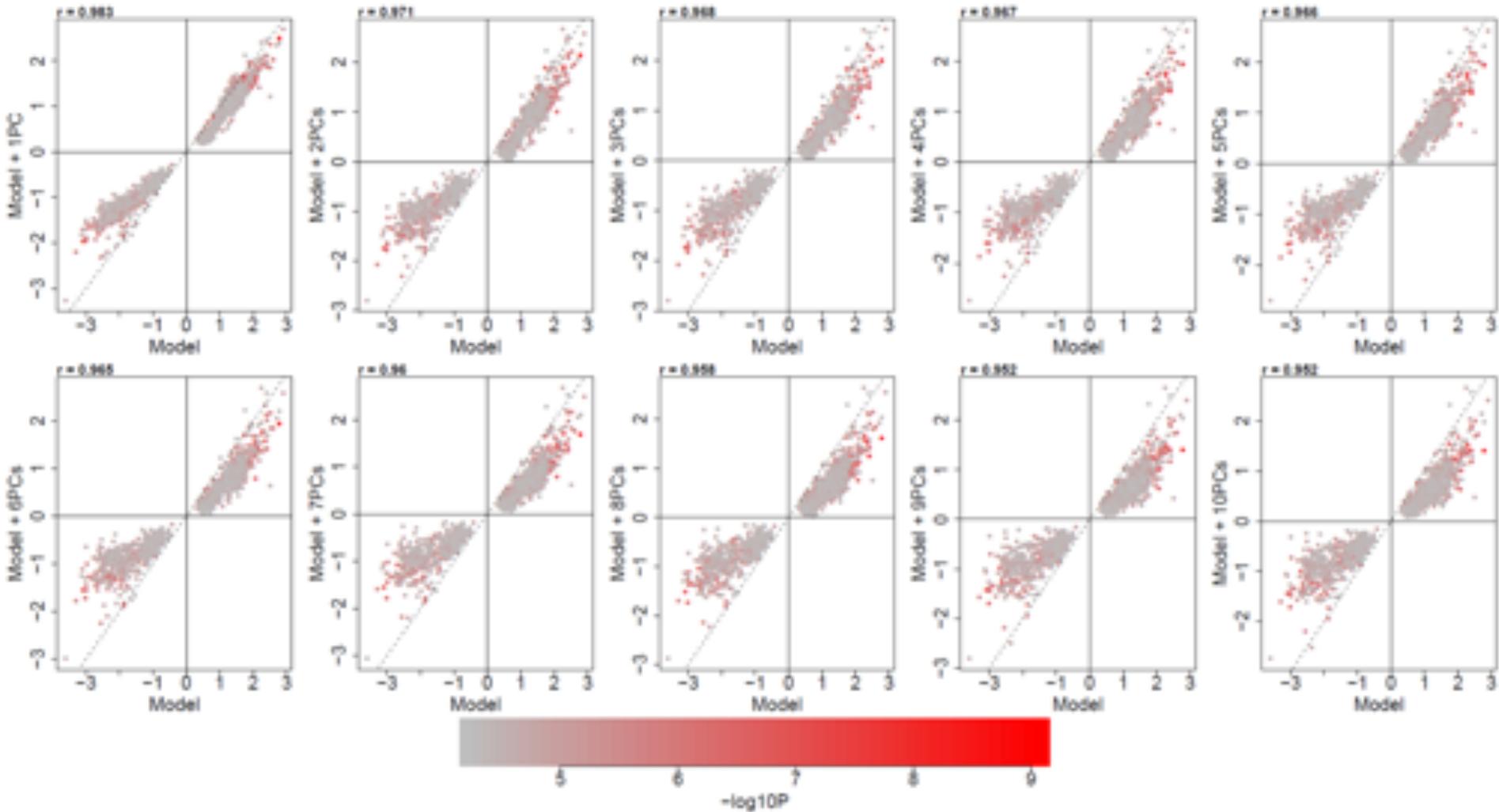


# Blood SZ EWAS – Phase 1 (UCL) – model controlling for sex, age, cell counts, smoking

25 DMPs with  $P < 1 \times 10^{-7}$   
1,223 DMPs with  $P < 5 \times 10^{-5}$



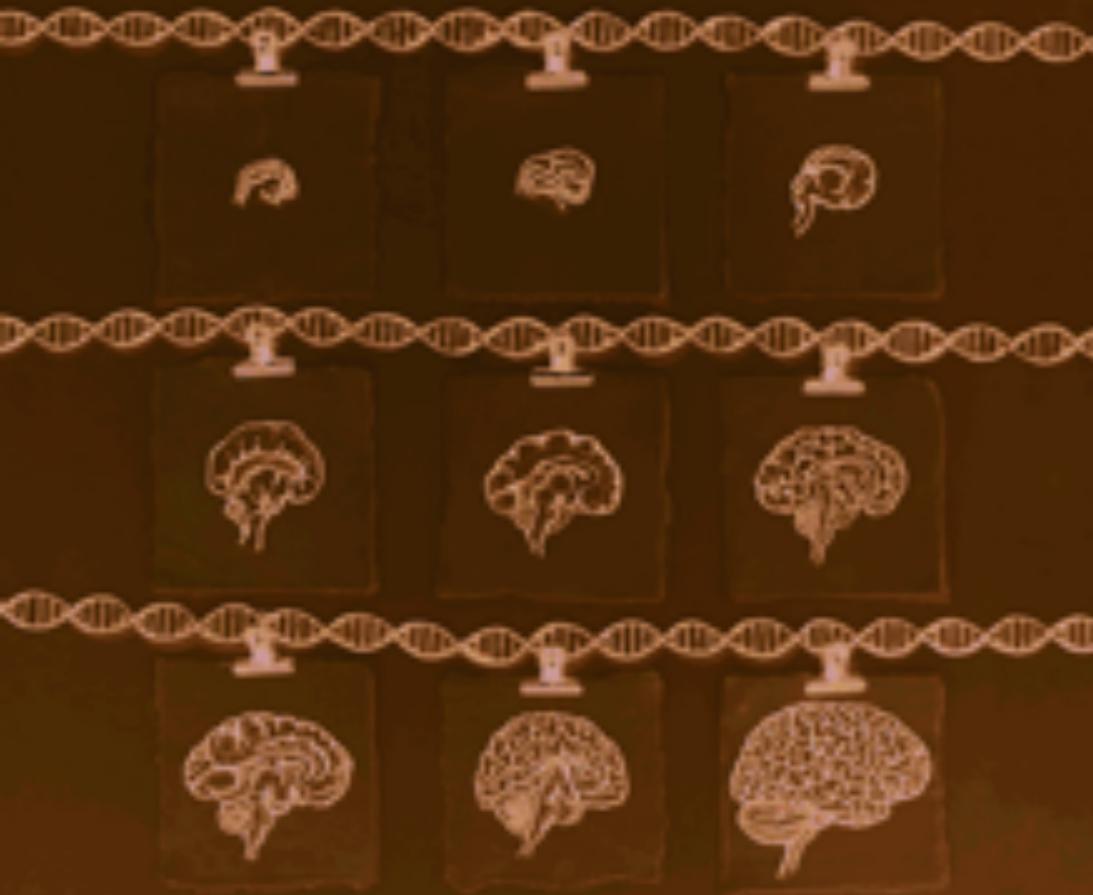
# Using principal components to test for additional confounding in data



# nature neuroscience

VOLUME 19 NUMBER 1 JANUARY 2016  
www.nature.com/natureneuroscience

Developmental epigenomics of schizophrenia  
Oligodendrocyte death triggers autoimmunity  
Neuromarker of sustained attention



166 human fetal brain samples with genetic and DNA methylation data

56-166 days post-conception

Prefrontal cortex, striatum and cerebellum samples from adult brain

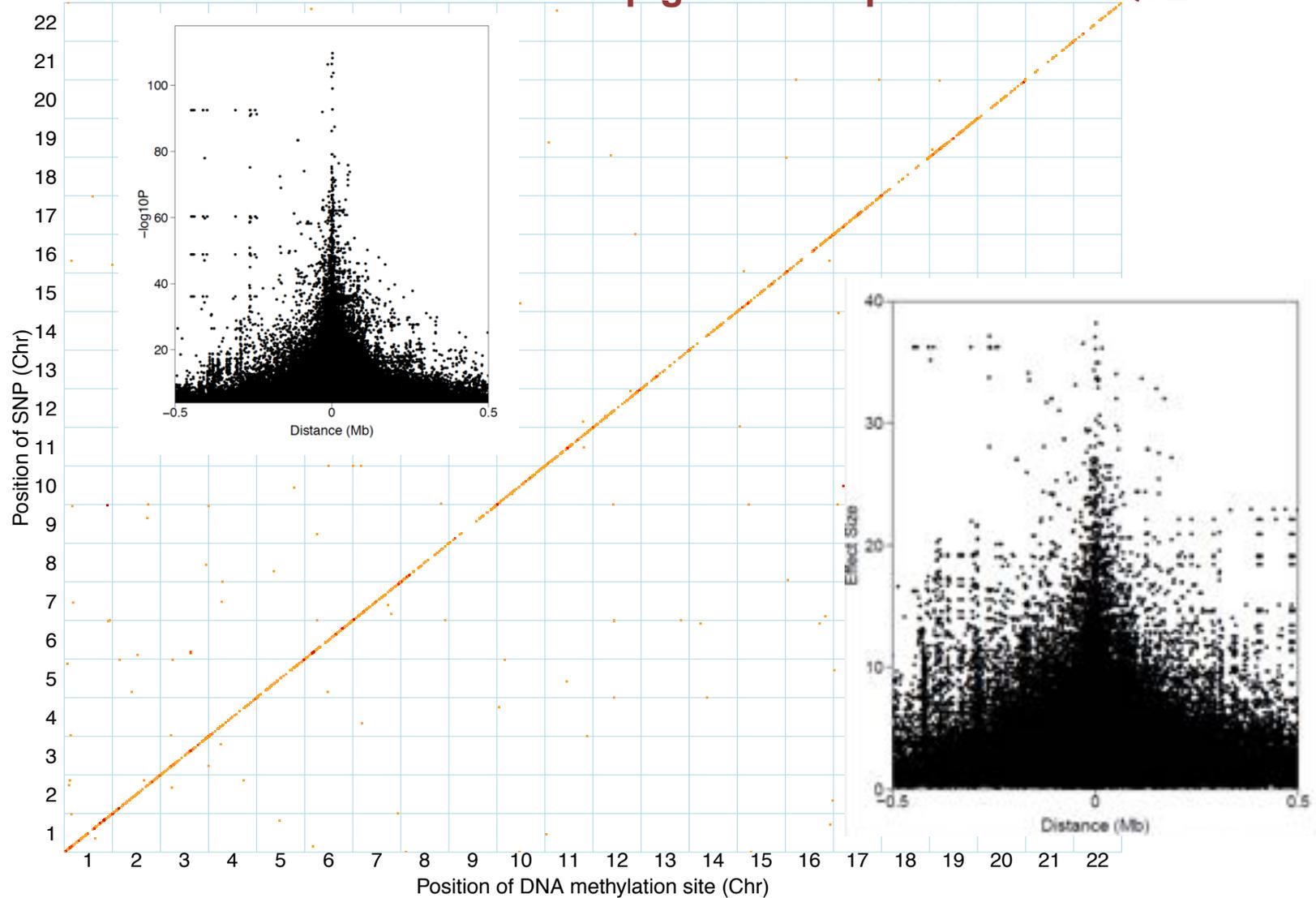
16,809 mQTLs at a conservative Bonferroni-corrected significance threshold of  $P < 3.69 \times 10^{-13}$

Imputation to 1,000 genomes project identified an additional 256,040 mQTLs

Median DNA methylation change per allele across all identified mQTLs = 6.69% (interquartile range = 3.17%-8.96%)

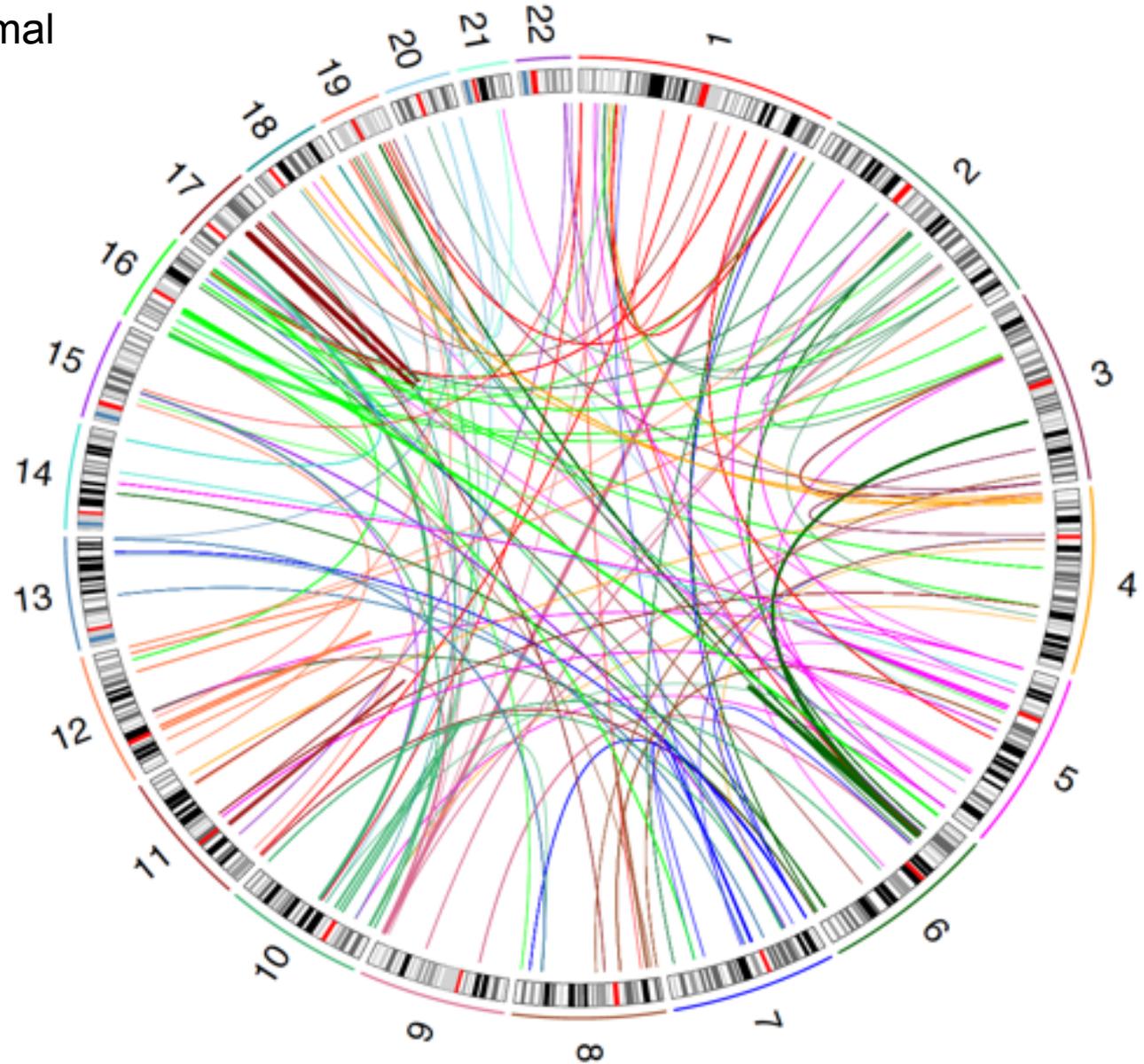
# The majority of fetal brain mQTLs (96.3%) involve SNPs and DNA methylation sites on the same chromosome

[epigenetics.iop.kcl.ac.uk/mQTL](http://epigenetics.iop.kcl.ac.uk/mQTL)



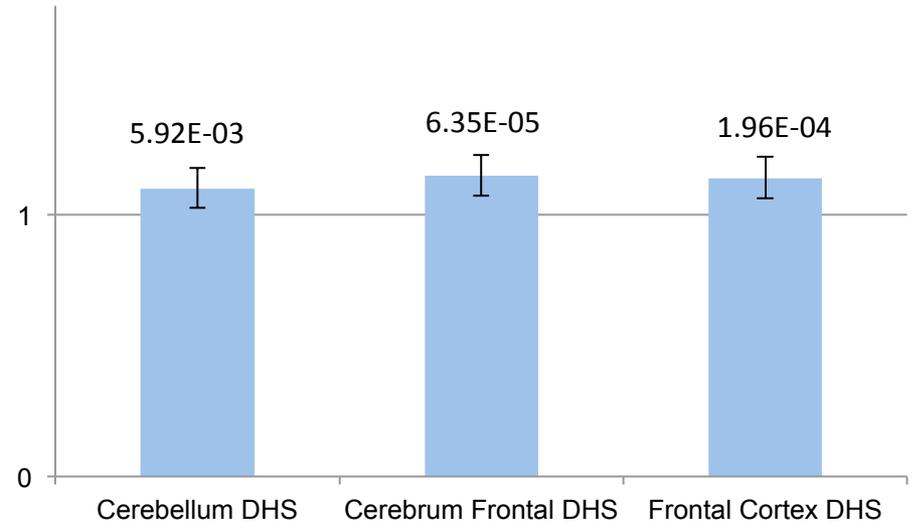
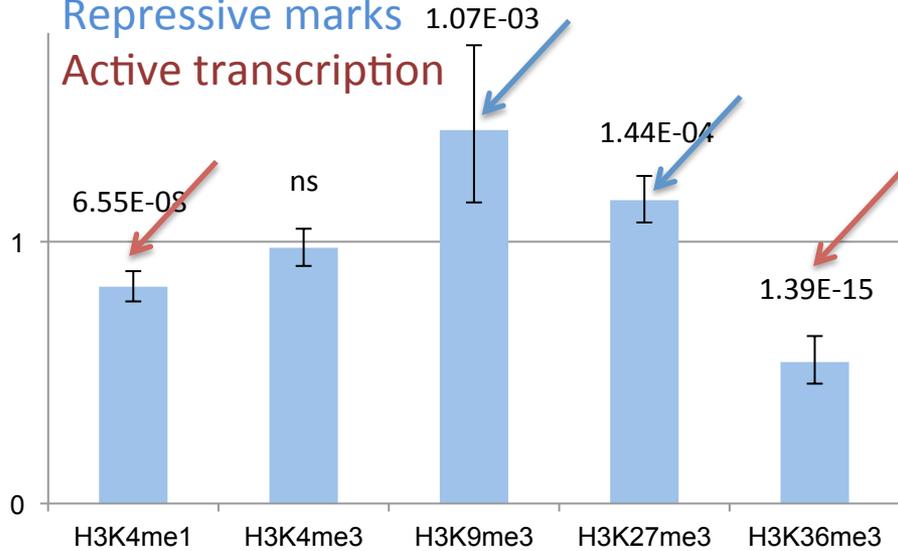
# Trans-mQTLs in the developing human brain

~700 inter-chromosomal  
(*trans*) mQTLs ( $P < 3.69 \times 10^{-13}$ )

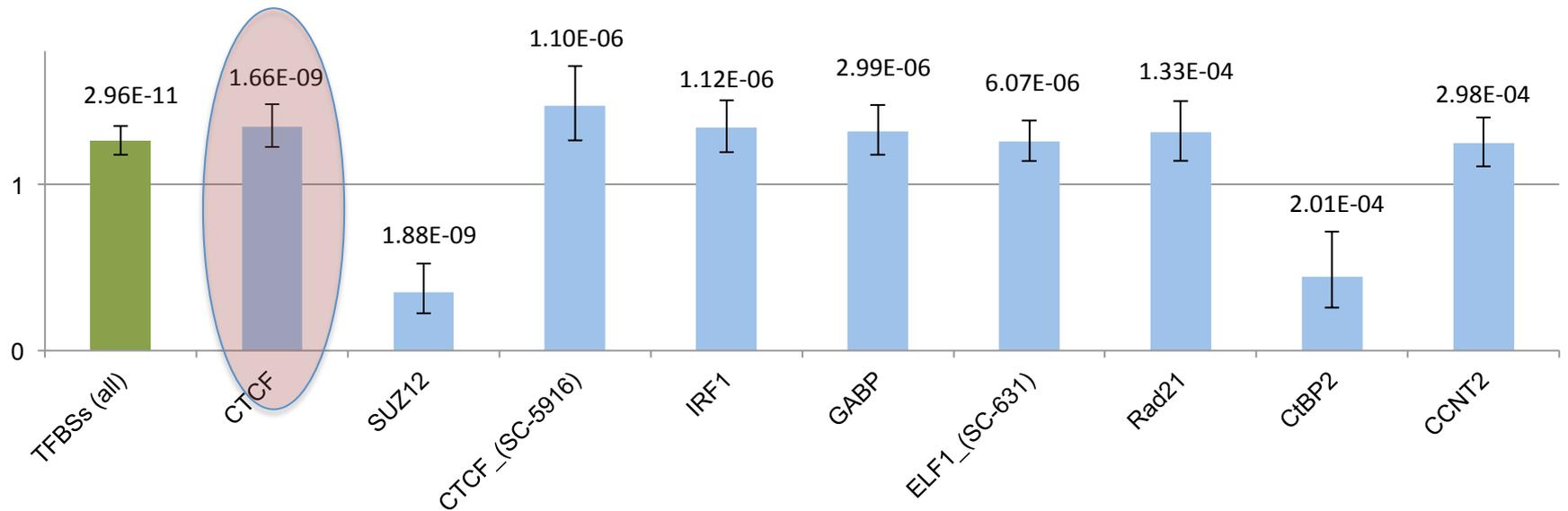


Repressive marks

Active transcription



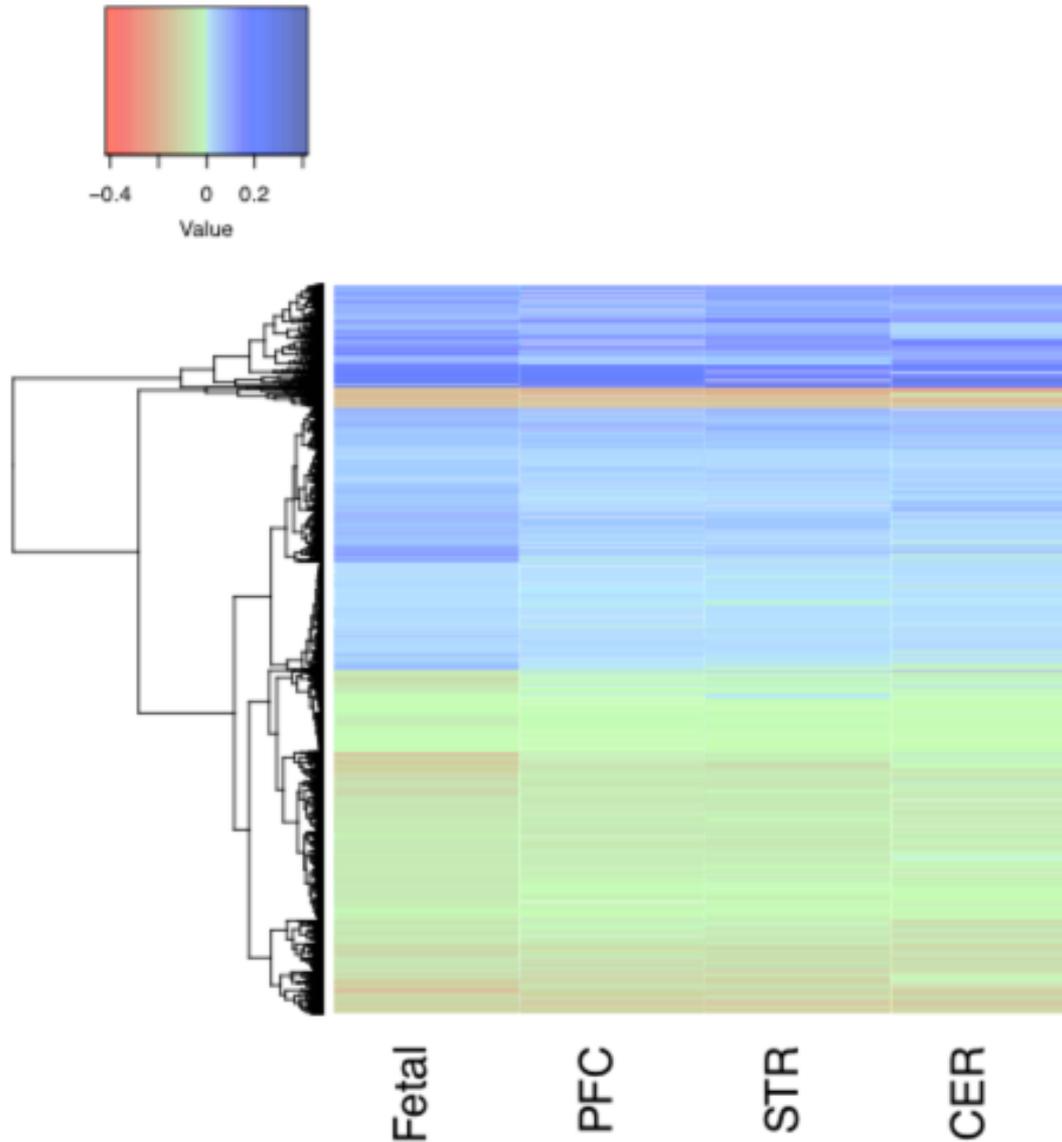
Fetal Brain ChIP-seq



Brain DHS sites

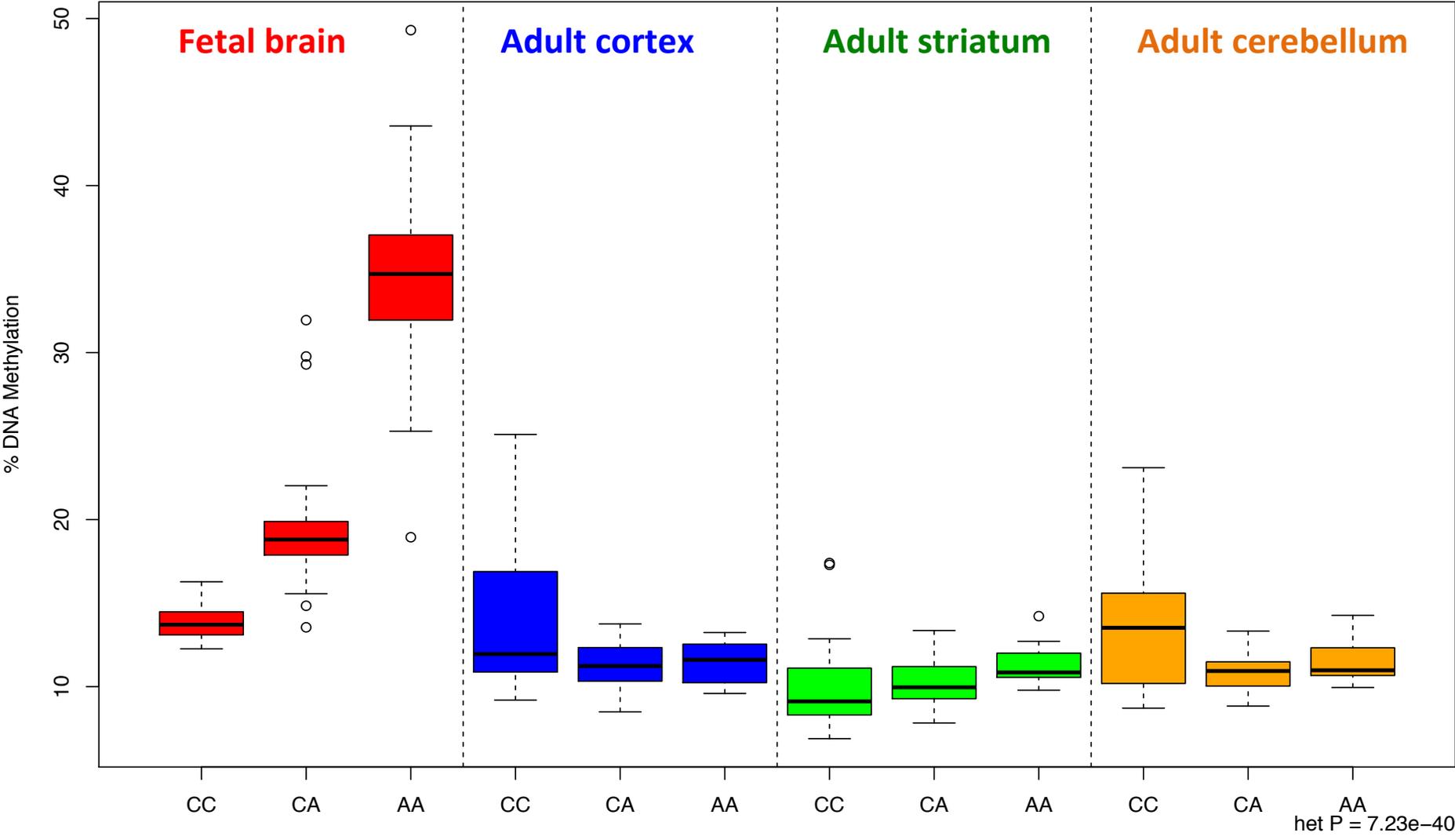
ENCODE TFBSs

# There is a high-correlation of mQTL effects between fetal brain and adult brain regions...



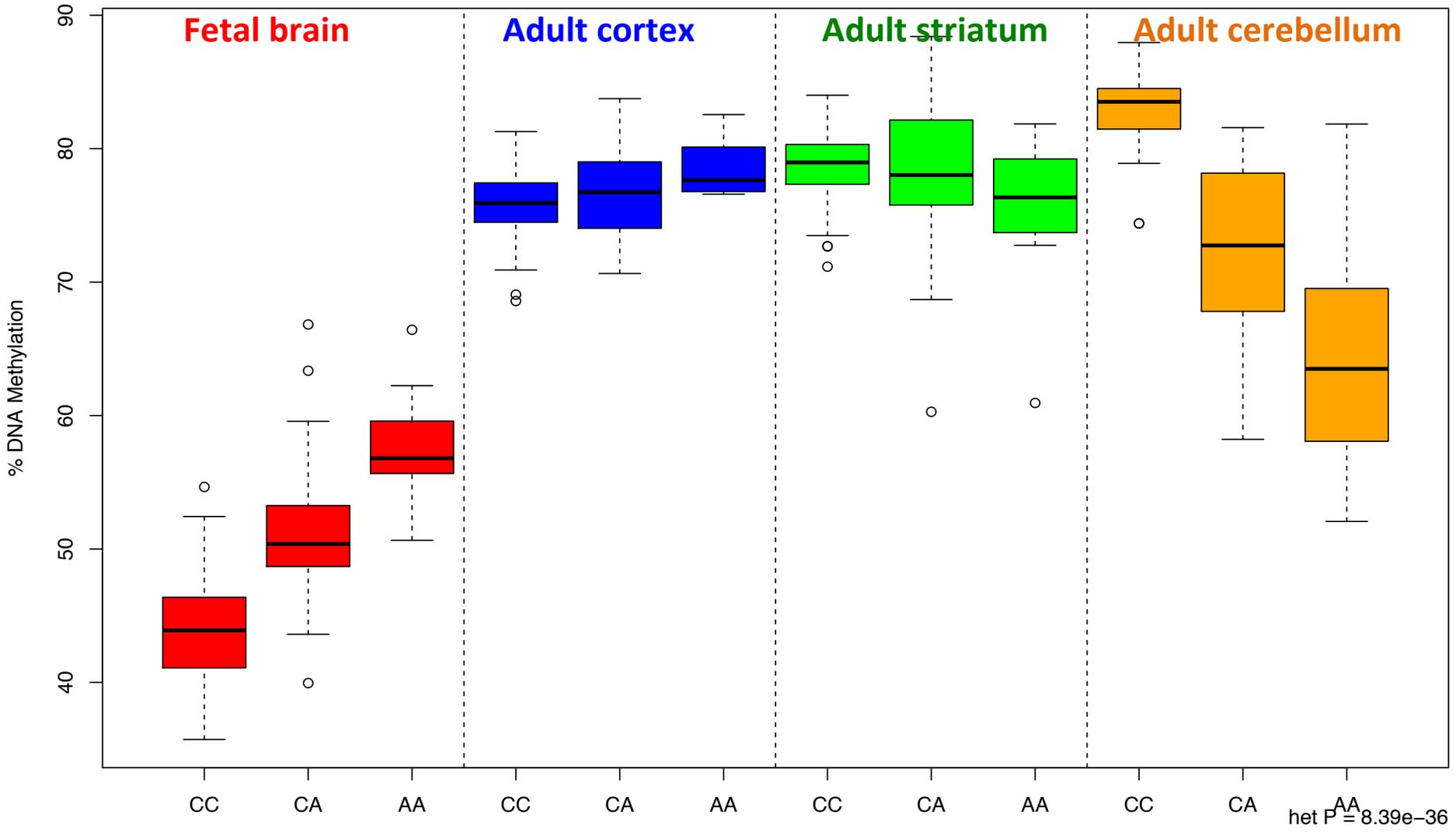
# Examples of fetal-specific genetic effects on regulatory variation

rs10447470 – cg07900658



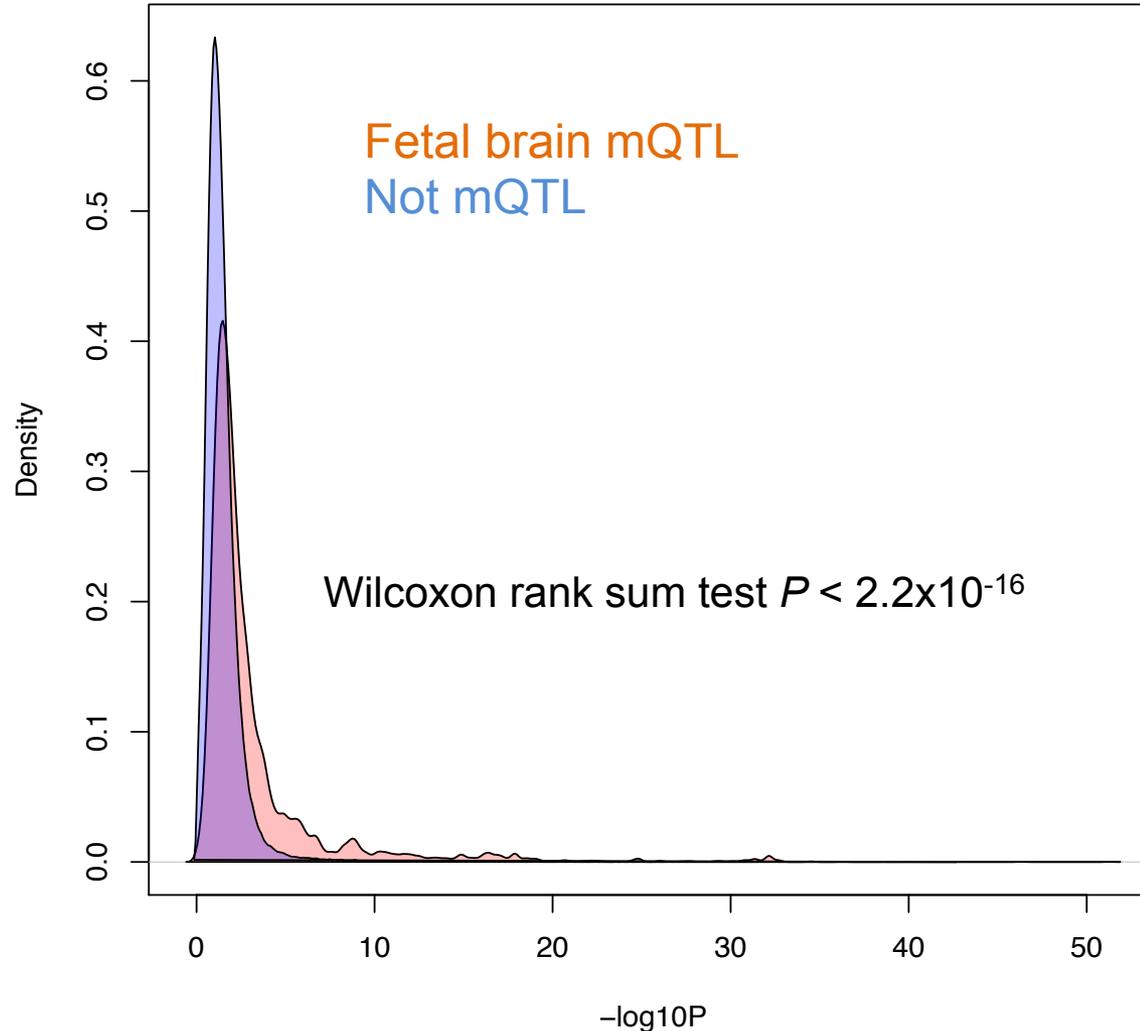
# ...and opposite-effect effects across tissues and developmental stage.

rs2108854 – cg21577356



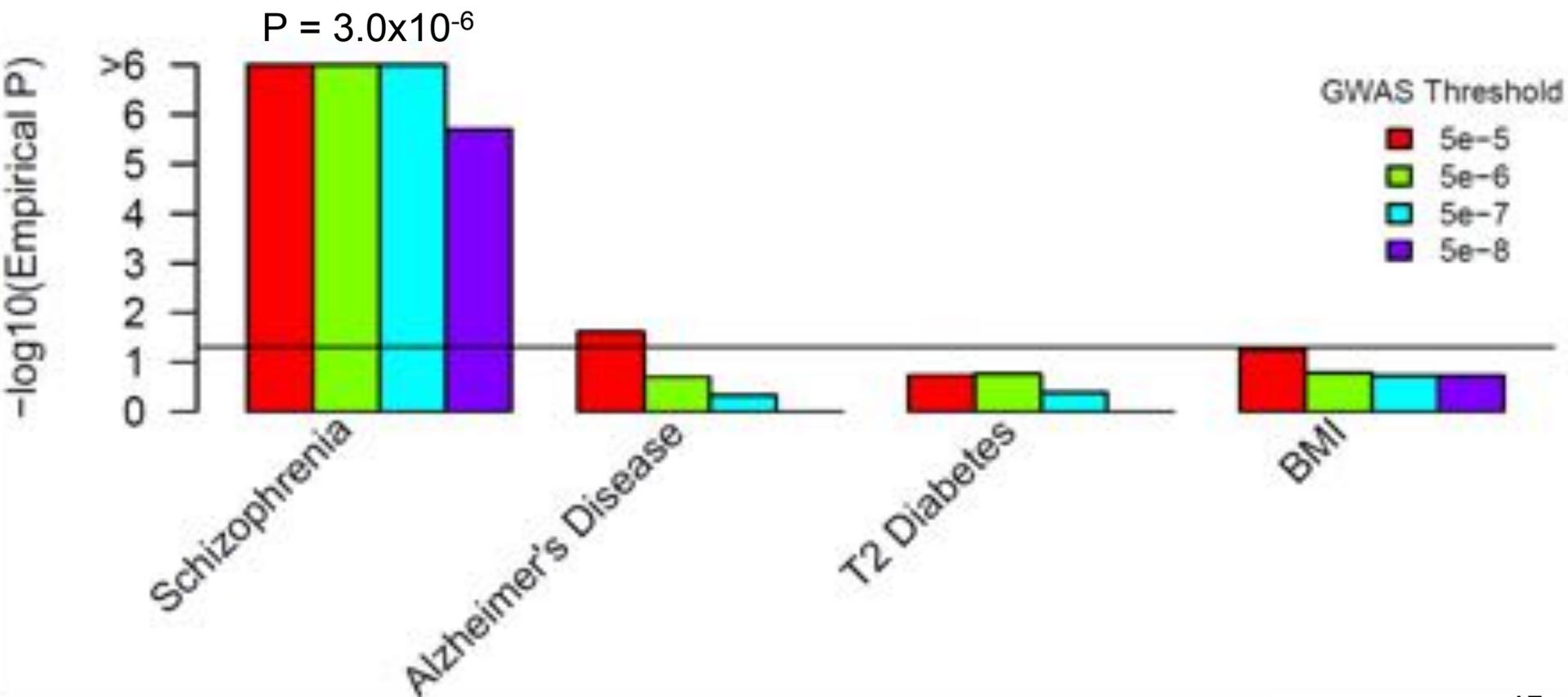
het  $P = 8.39e-36$

# SNPs associated with DNA methylation are more significantly associated with gene expression than non-mQTL variants.

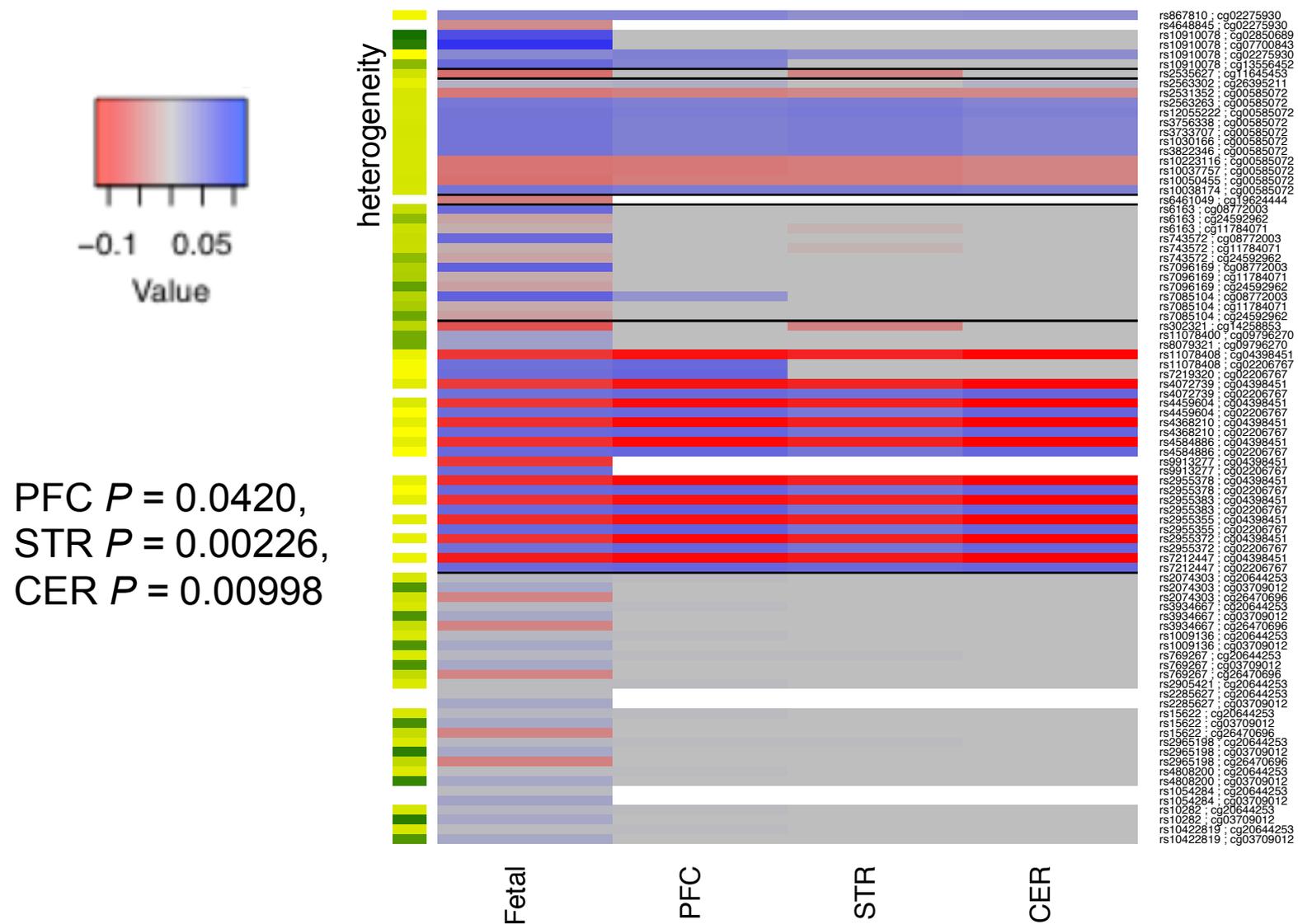


*eQTL data from  
BRAINIAC (Mike  
Weale)*

# Highly-significant enrichment of genome-wide significant schizophrenia risk variants amongst fetal brain mQTLs

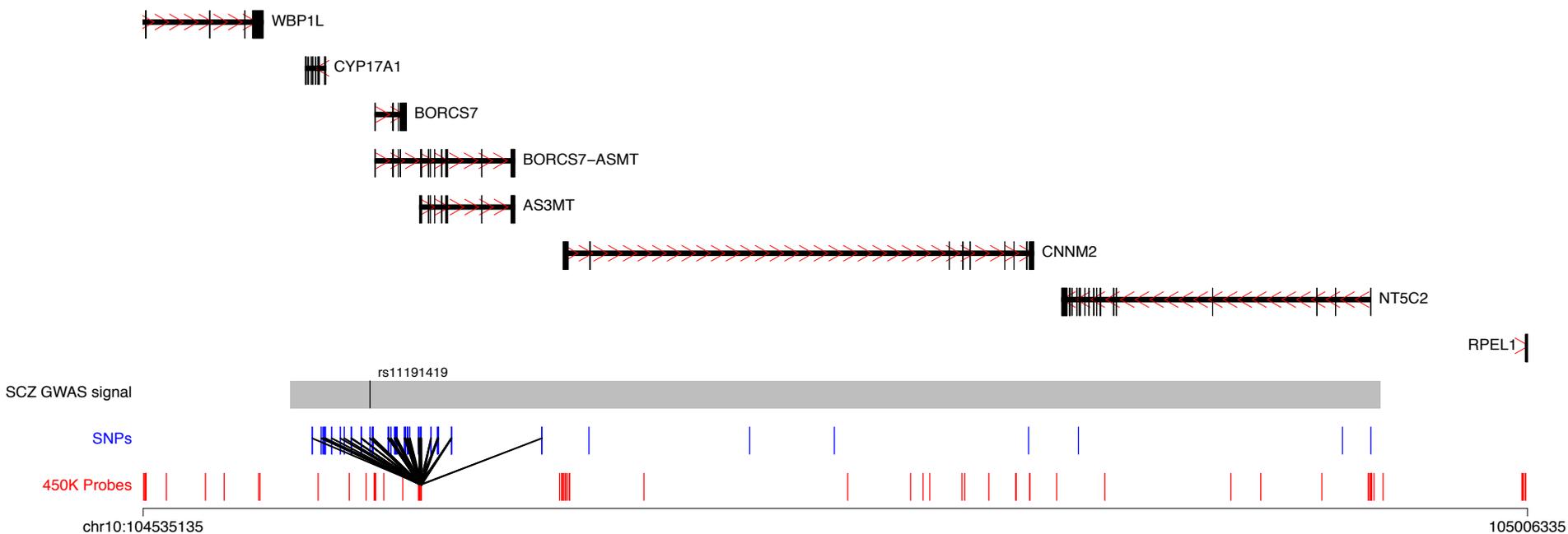


# Fetal mQTLs in schizophrenia-associated regions have larger effects on DNA methylation during neurodevelopment than the in adult brain

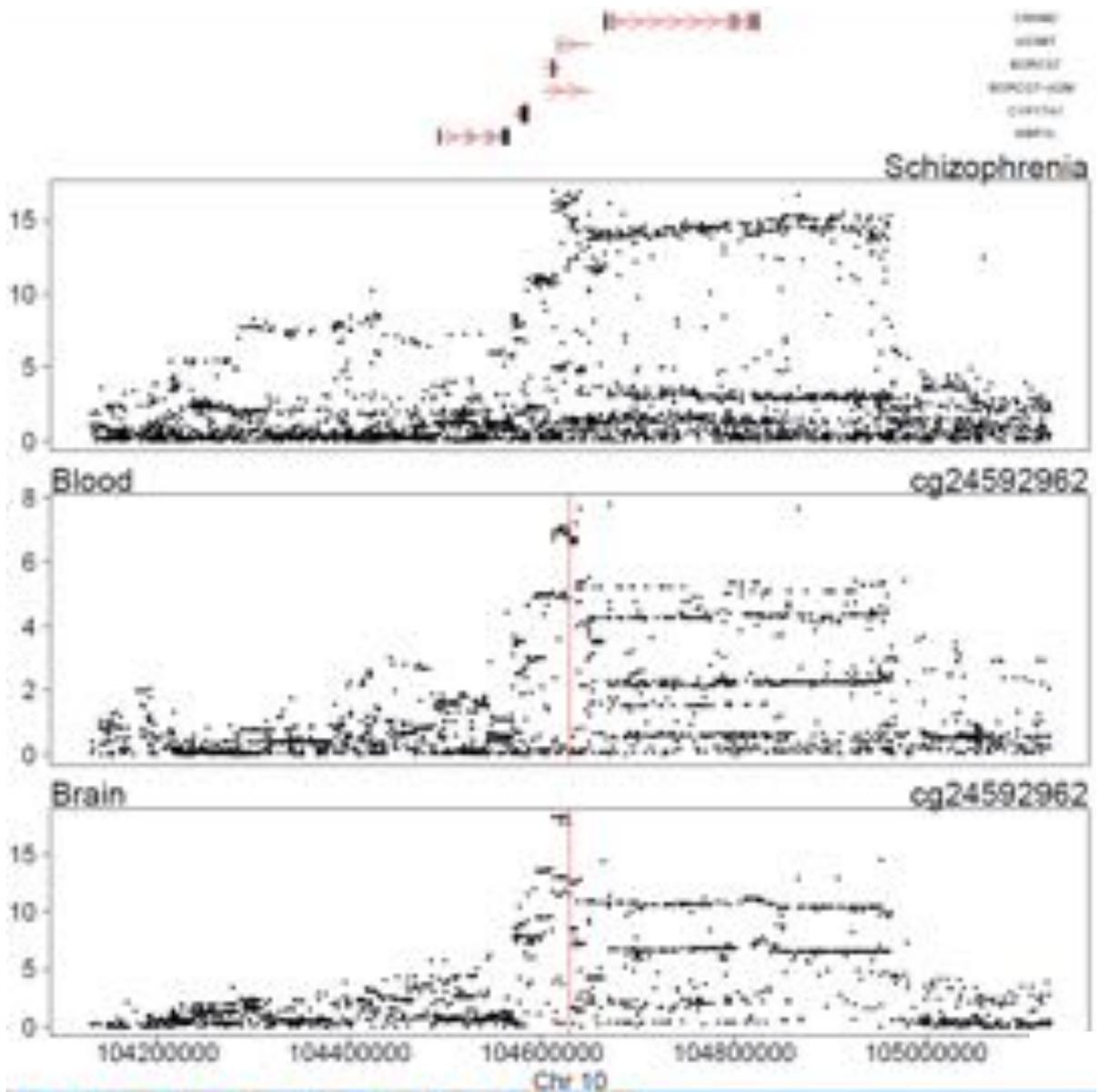


# Bayesian co-localization analysis across 105 autosomal regions associated with schizophrenia

- posterior probabilities for 65 regions (involving 296 DNA methylation sites in 306 pairs) were supportive of a co-localized association signal for both schizophrenia and DNA methylation in that region
- 26 of these pairs (covering 15 regions associated with schizophrenia) strongly supportive for both schizophrenia and DNA methylation being associated with the same causal variant

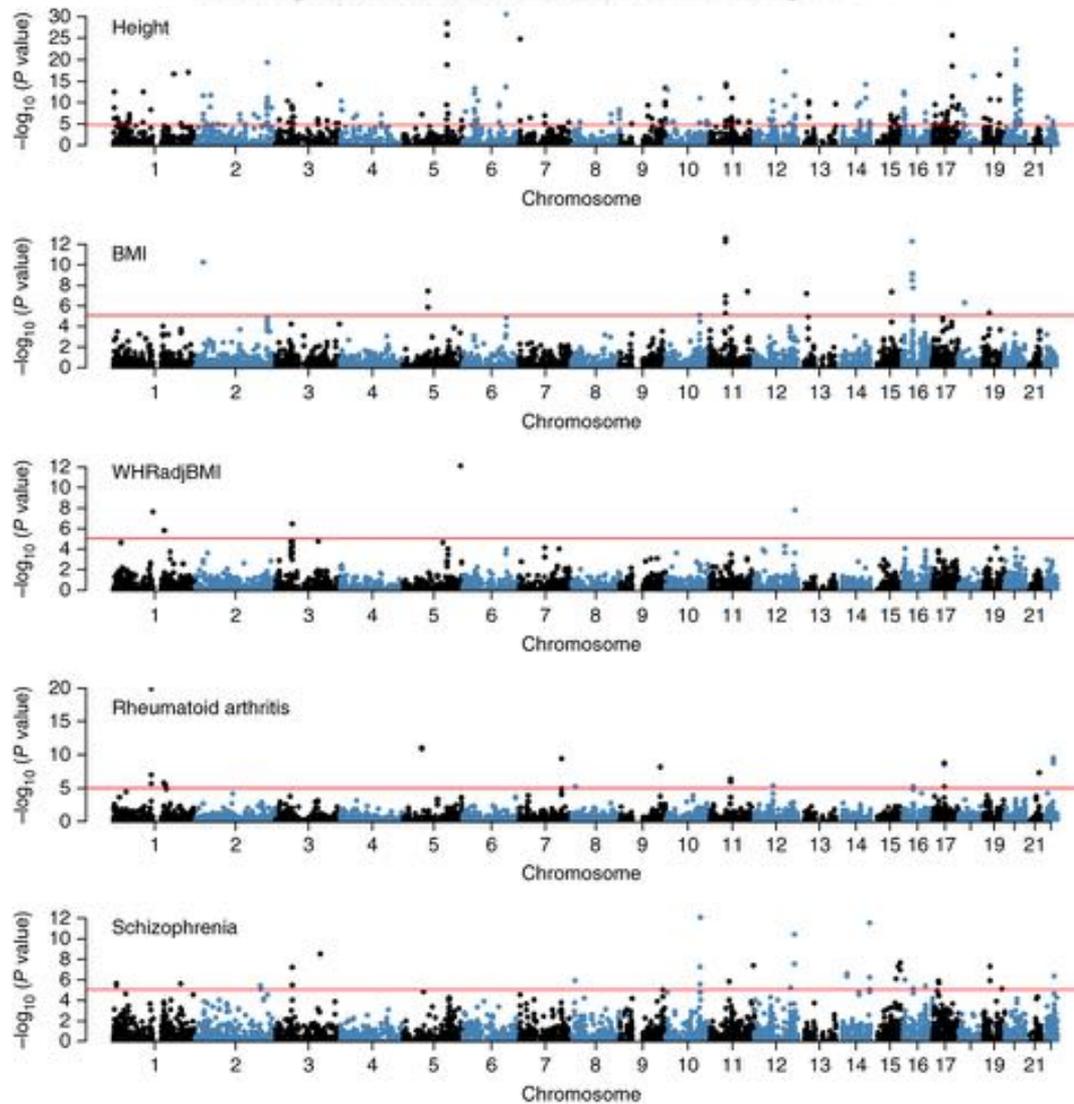


# Bayesian co-localization approaches to identify variants associated with both disease and genomic regulation

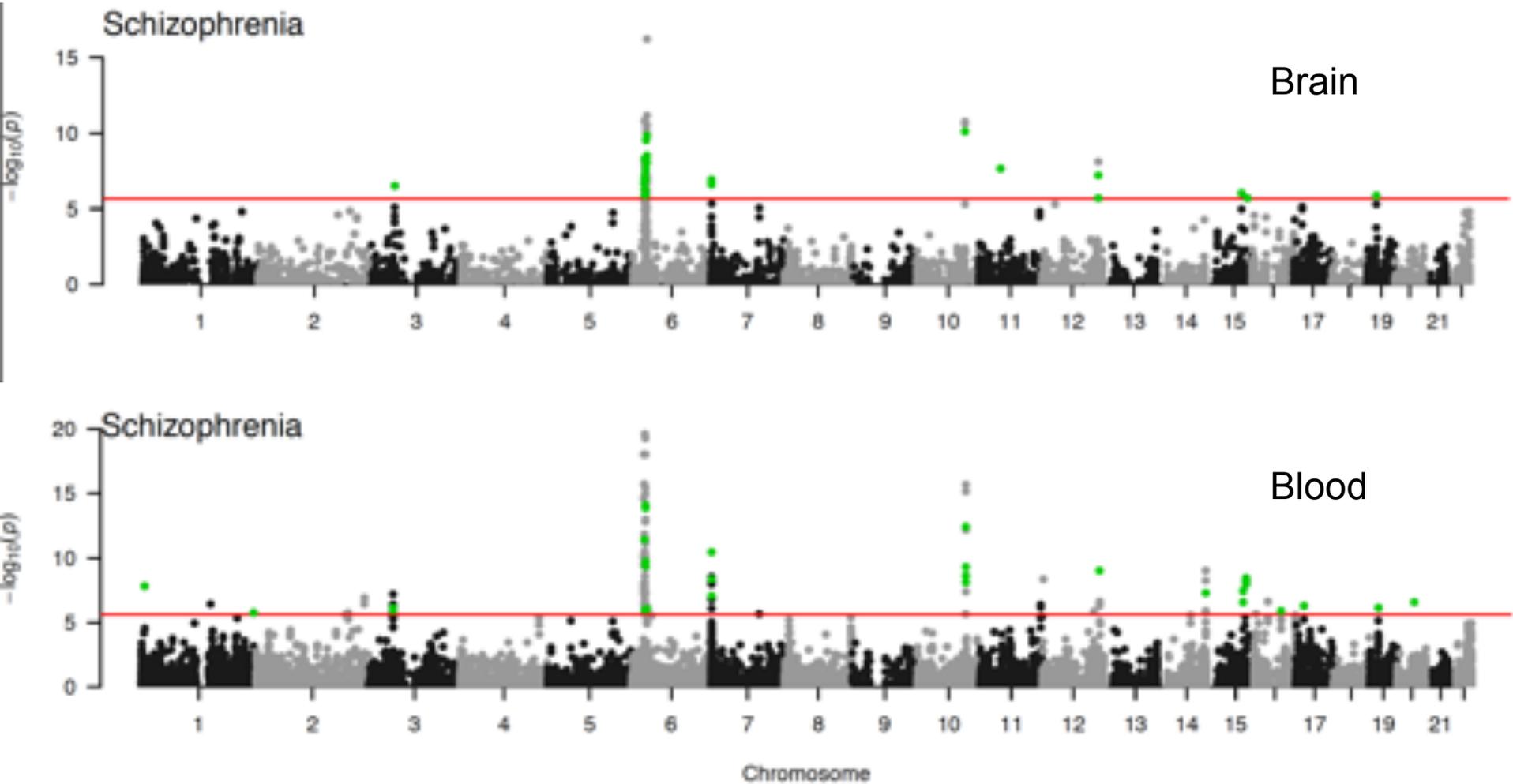


### Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets

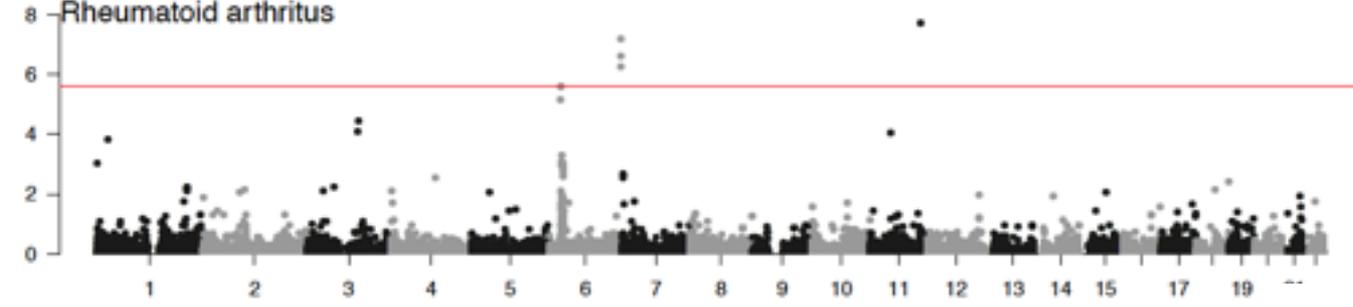
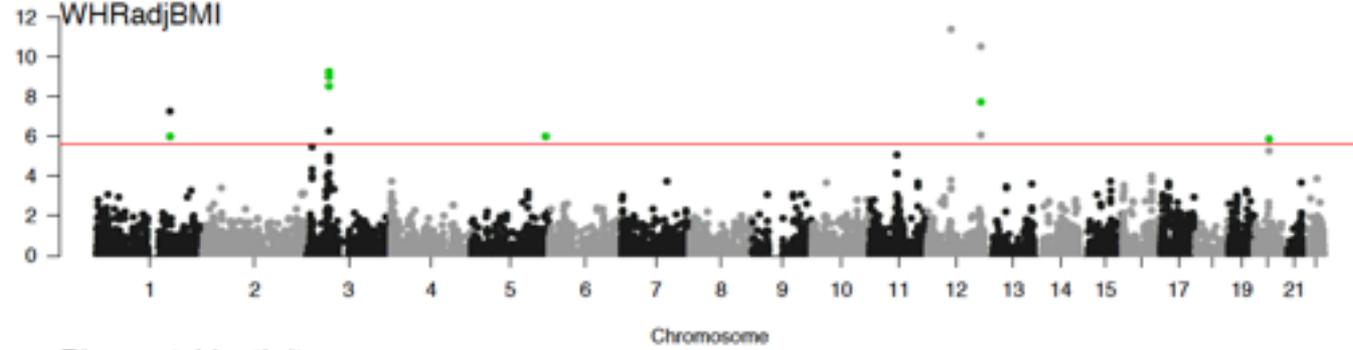
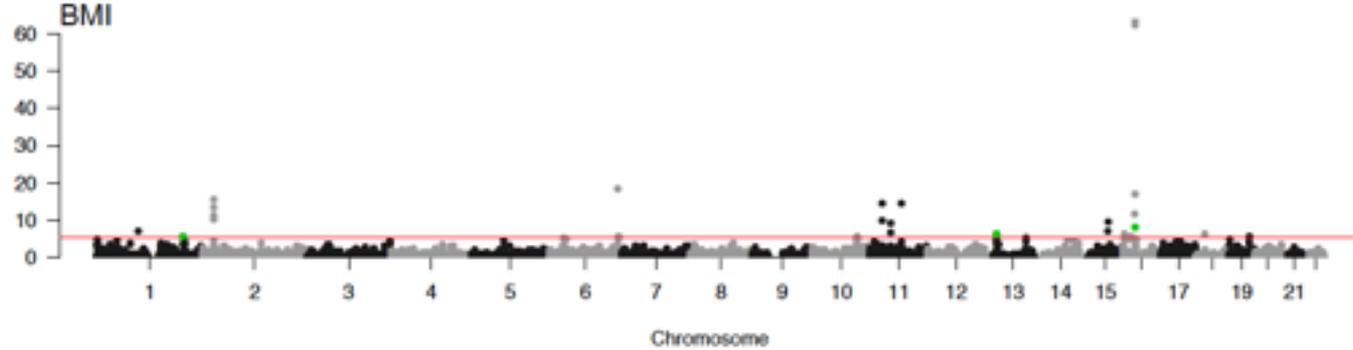
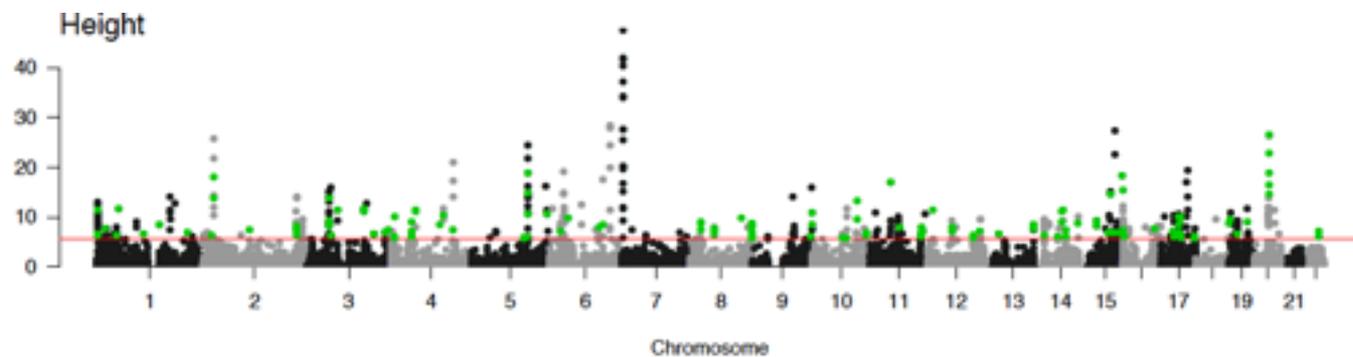
Zhiling Chen<sup>1</sup>, Peter Chang<sup>1</sup>, Xia Hu<sup>1</sup>, Andrew Baheti<sup>1</sup>, Matthew B Robinson<sup>1</sup>, Joseph E Powell<sup>1,2</sup>, Grant W Montgomery<sup>1</sup>, Michael B Goddard<sup>1,3</sup>, Naomi R Wray<sup>1</sup>, Peter W Visscher<sup>1,4</sup> & Dan Yang<sup>1\*</sup>



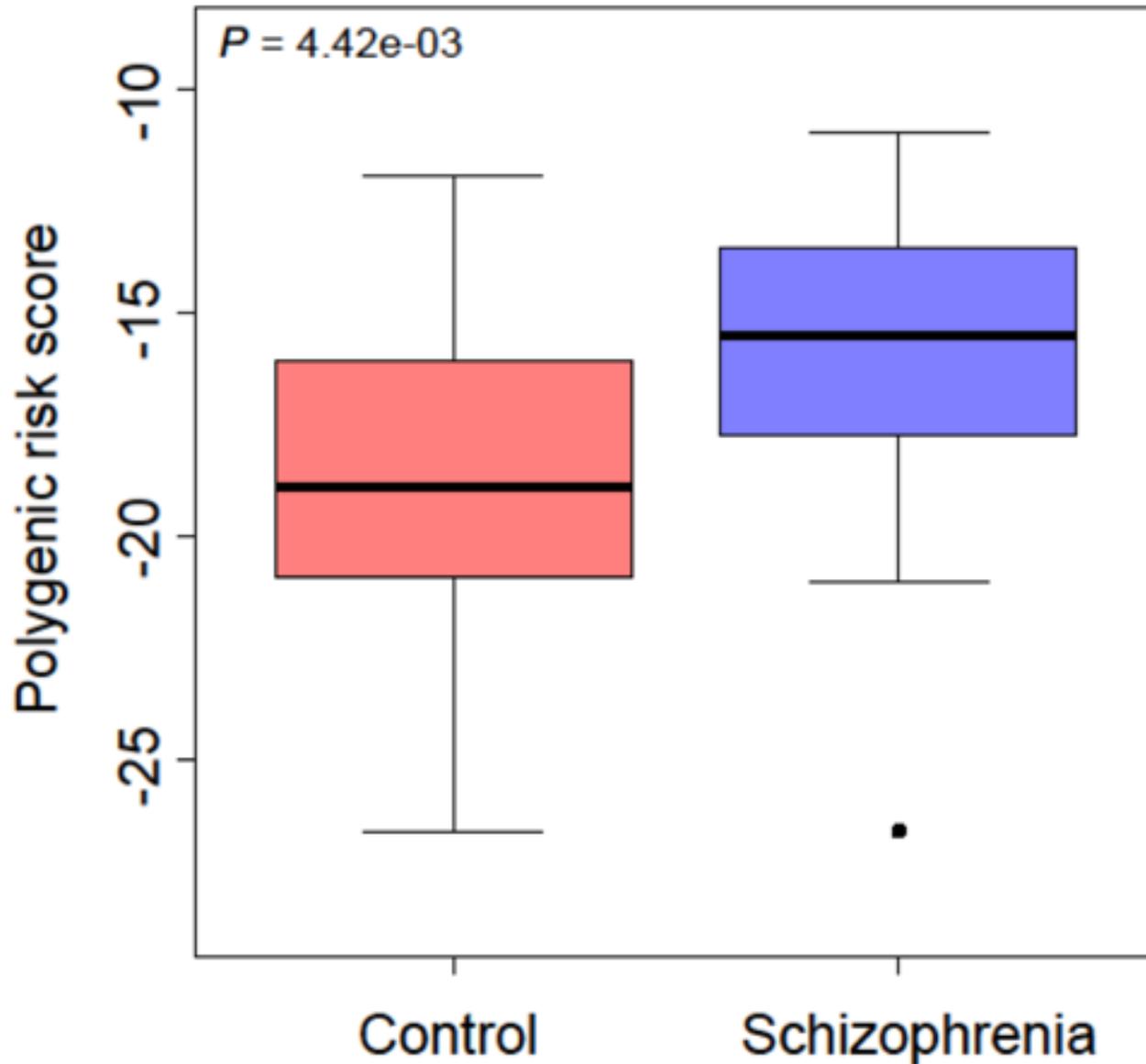
# SMR analysis for SZ / DNA methylation



Green = probes that pass both steps of the SMR analysis (pleiotropy not LD)

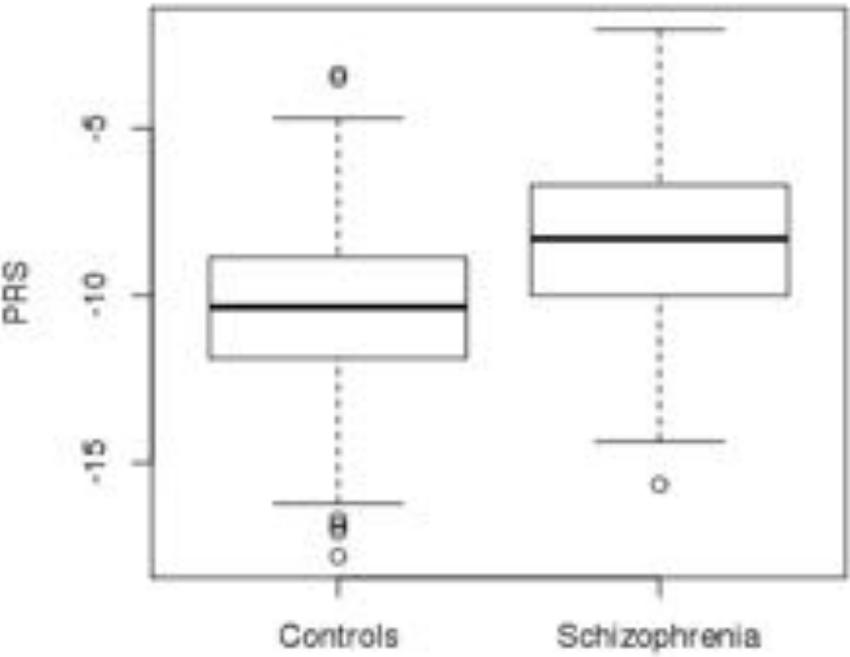


# Increased polygenic risk burden in schizophrenia brain samples



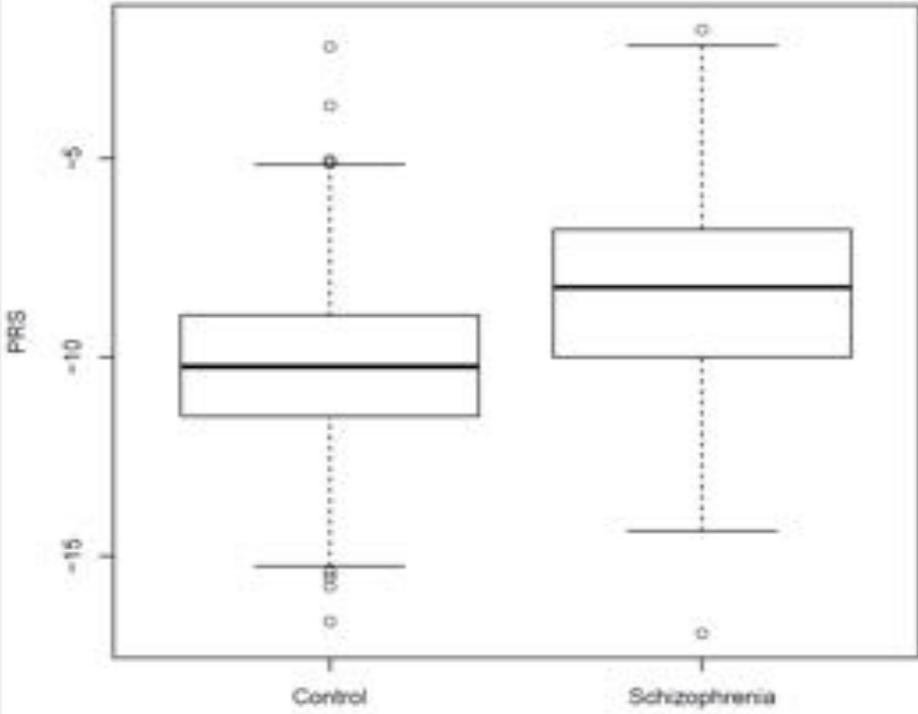
# Increased polygenic risk burden in schizophrenia blood samples

Phase 1 - UCL



$P = 3.34 \times 10^{-27}$

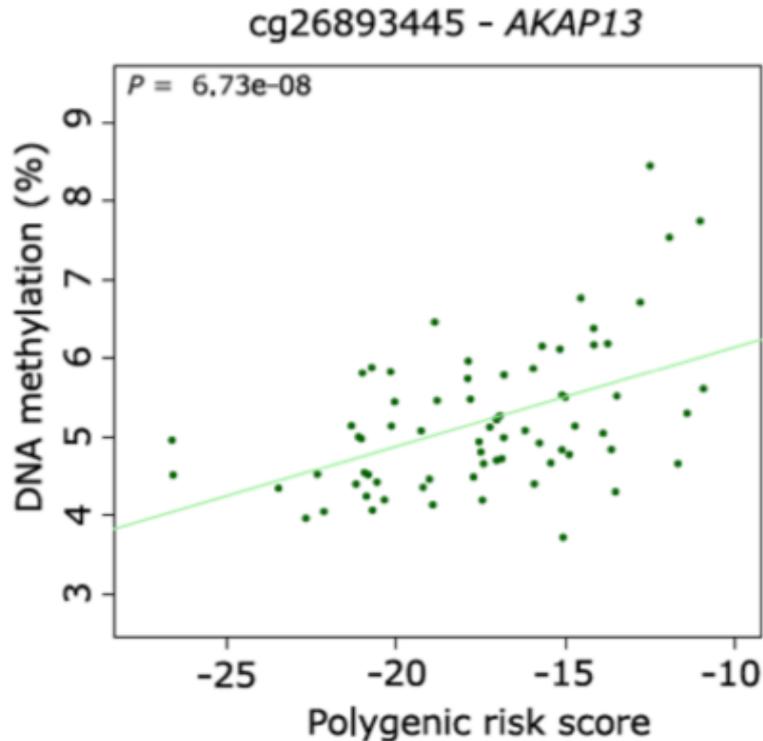
Phase 2 - Aberdeen



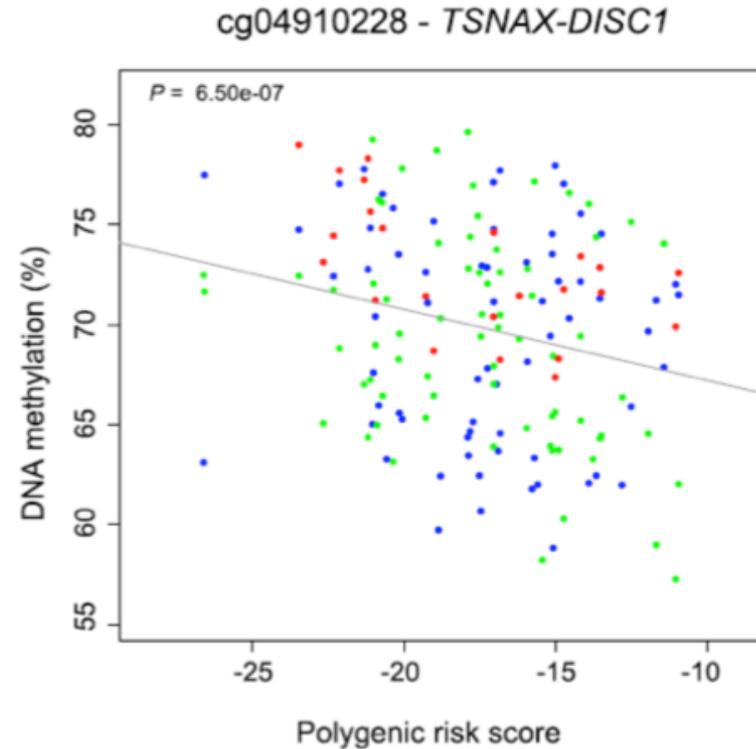
$P = 2.09 \times 10^{-31}$

# Examples of PRS-associated DNA methylation in brain

## Striatum

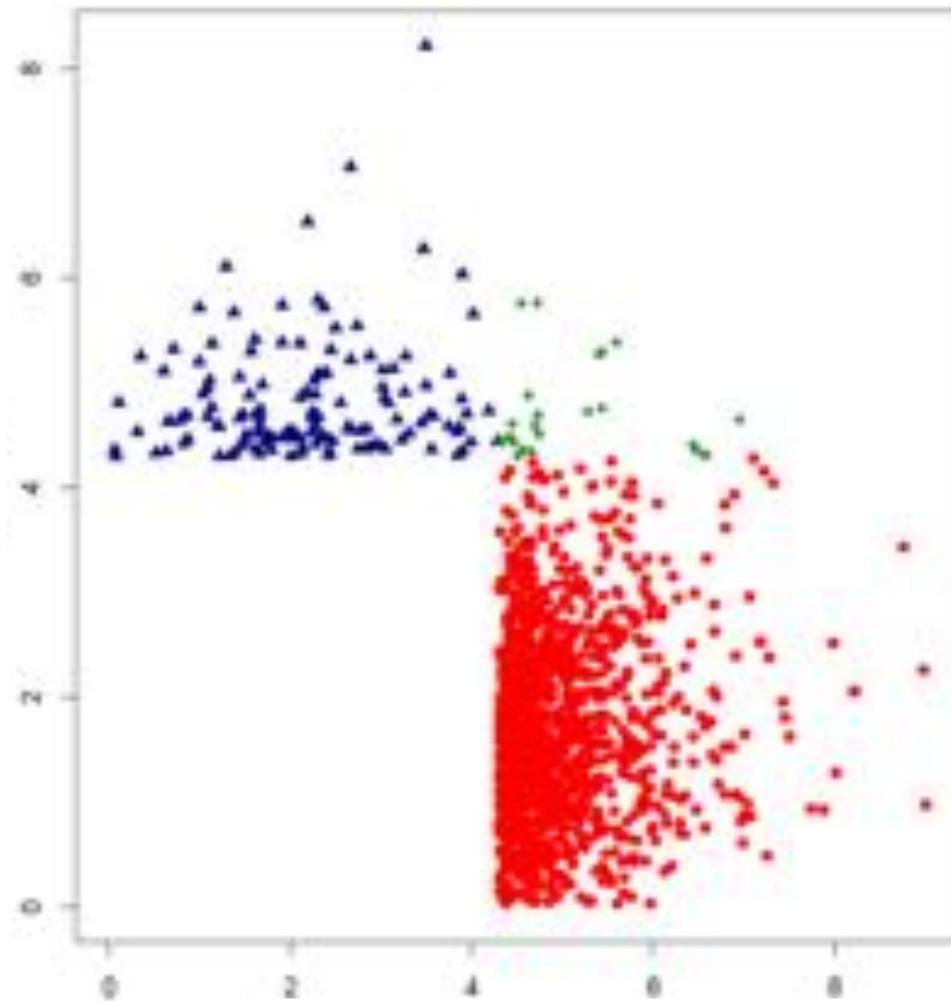


## Cross-brain region model



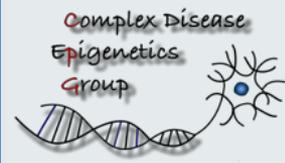
# DMPs associated with schizophrenia diagnosis are distinct from those associated with polygenic burden

**Polygenic  
risk score  
EWAS**



PRS-associated  
variation does not  
appear to be  
mediated by mQTL  
effects

**Schizophrenia EWAS**



## Schizophrenia project acknowledgments

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