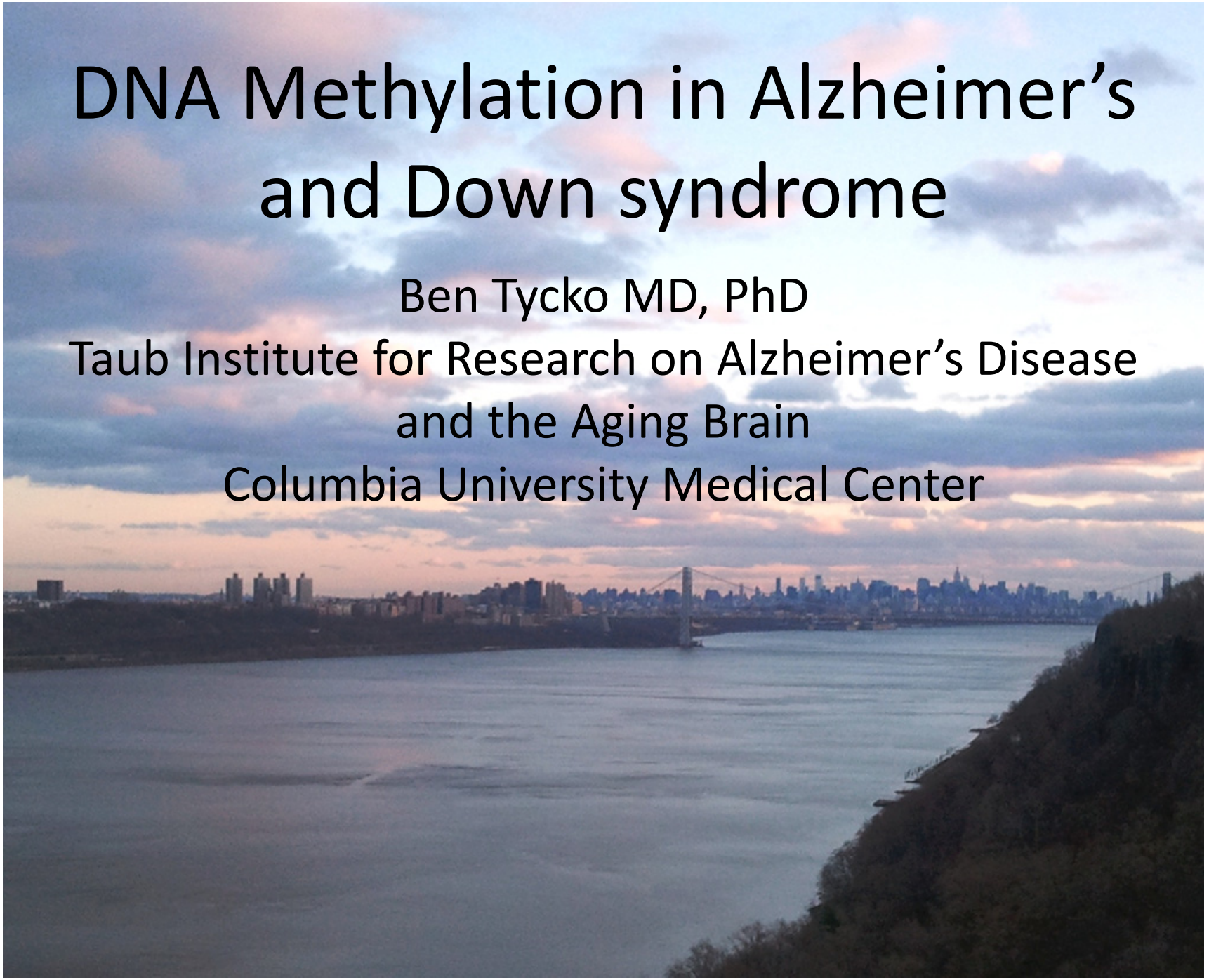
DNA Methylation in Alzheimer's and Down syndrome

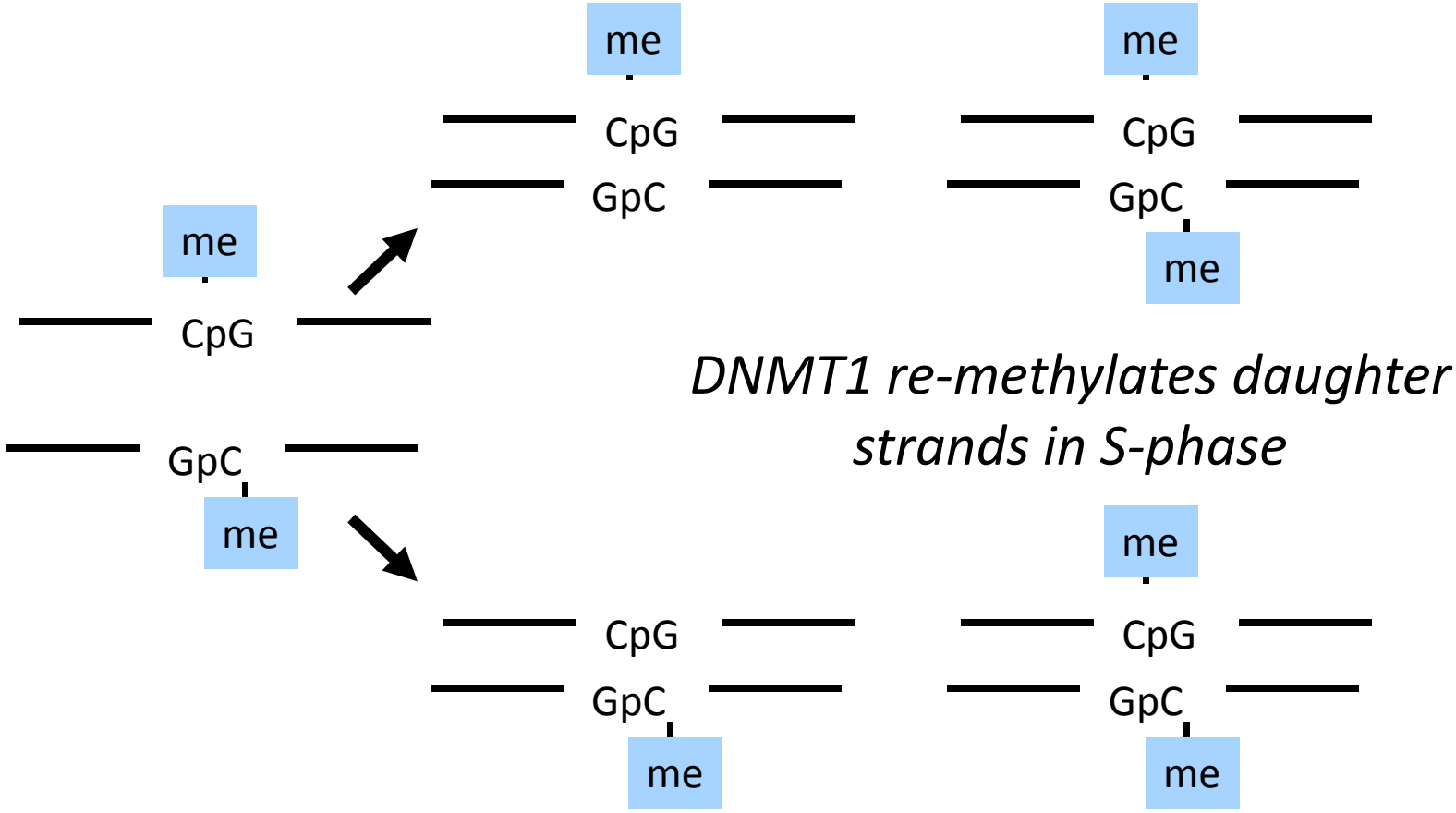
Ben Tycko MD, PhD
Taub Institute for Research on Alzheimer's Disease and the Aging Brain
Columbia University Medical Center



Why study DNA methylation?

- Promoter CpG methylation silences gene expression; methylation elsewhere can activate genes.
- Methylation is perturbed in disease – best studied in cancer but now of interest (NIH Roadmap) in many other diseases – especially neurological ones.
- Methylation can be manipulated by drugs and diet (5-aza-dC; folate)
- Mapping CpG methylation can highlight genetic-epigenetic interactions – help to find disease risk loci.

DNA methylation: faithfully transmitted to daughter cells in proliferating tissues

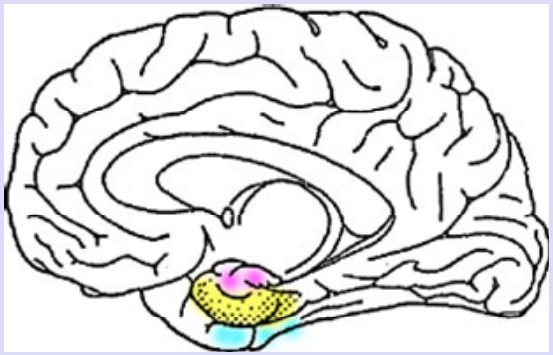


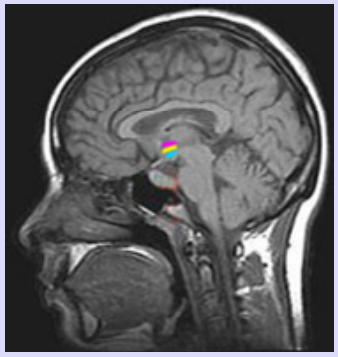
Changing DNA methylation: demethylation and *de novo* methylation

- Pre-existing methylation can be lost when DNA replicates in the absence of DNMT1
- *de novo* gain of methylation at previously unmethylated sequences is catalyzed by the other methyltransferase enzymes – DNMT3A and DNMT3B (DNMT3A is most important in the brain)

Could DNA methylation be "involved in AD"?

By Braak stage 2, tau tangles have accumulated further and have caused some neurons to burst apart and die. However, mental testing still shows minimal impairment. Tangles at this level or worse are found in the brains of about 60% of over-65s.
At this stage the tau tangles are much more extensive in the transitional entorhinal region (highlighted in yellow) and have begun to kill neurons here. This region is a "relay station" where sensory input is filtered before being stored in the memory. Meanwhile, in the brain's hippocampus (pink) and neo-cortex, tau protein is also beginning to aggregate but has not yet formed tangles.





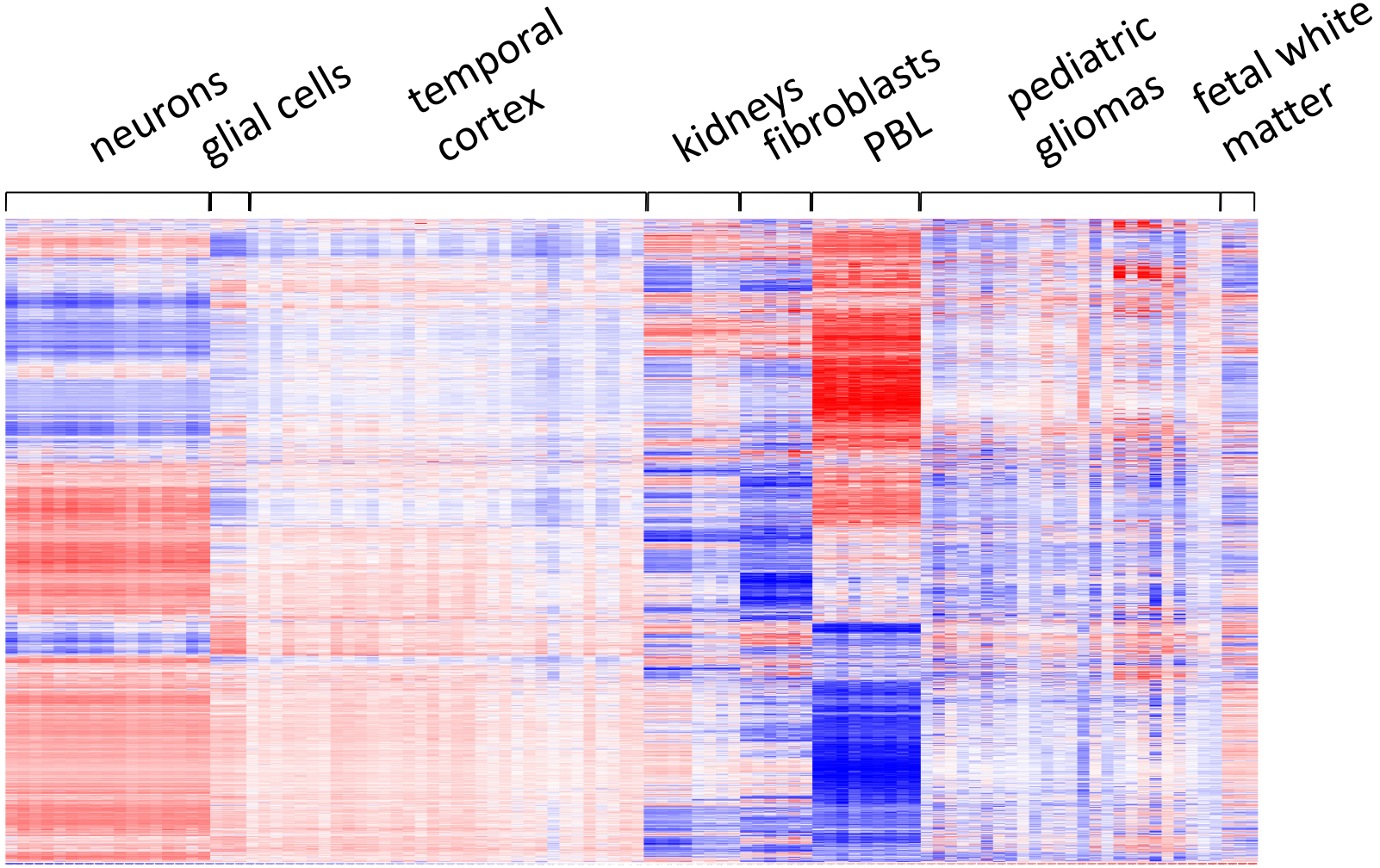
At Braak stage 2, **tau tangles** are causing cell death (yellow) in the *transitional entorhinal region*, a part of the brain that filters sensory input before passing it on to the memory. Tau protein is aggregating in the *hippocampus* (pink) which is crucial for memory, and in the *cortex* (blue) which is associated with conscious thought.

We know about toxic Abeta aggregates and tau tangles – why do we need another hypothesis?

Methods for profiling CpG methylation

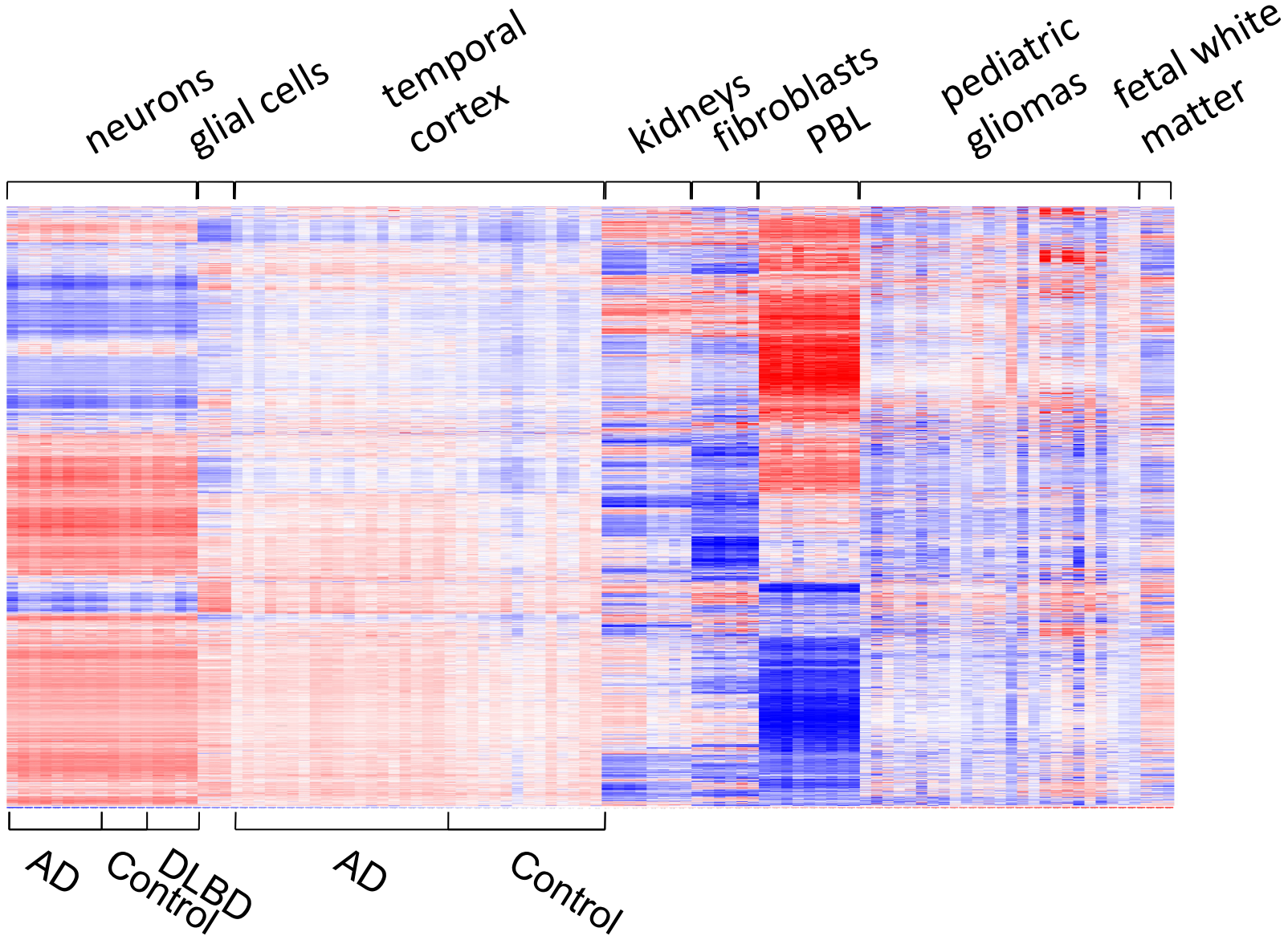
- MSNP and related methods (methyl-sensitive restriction enzymes; microarrays)
- MeDIP/MIRA (affinity capture of methylated DNA; microarrays or Nextgen sequencing)
- Illumina Infinium (bisulfite conversion; primer extension on Beadarrays)
- Nextgen bisulfite sequencing of targeted sequences or whole epigenomes

Promoter meth: *major differences among tissues*

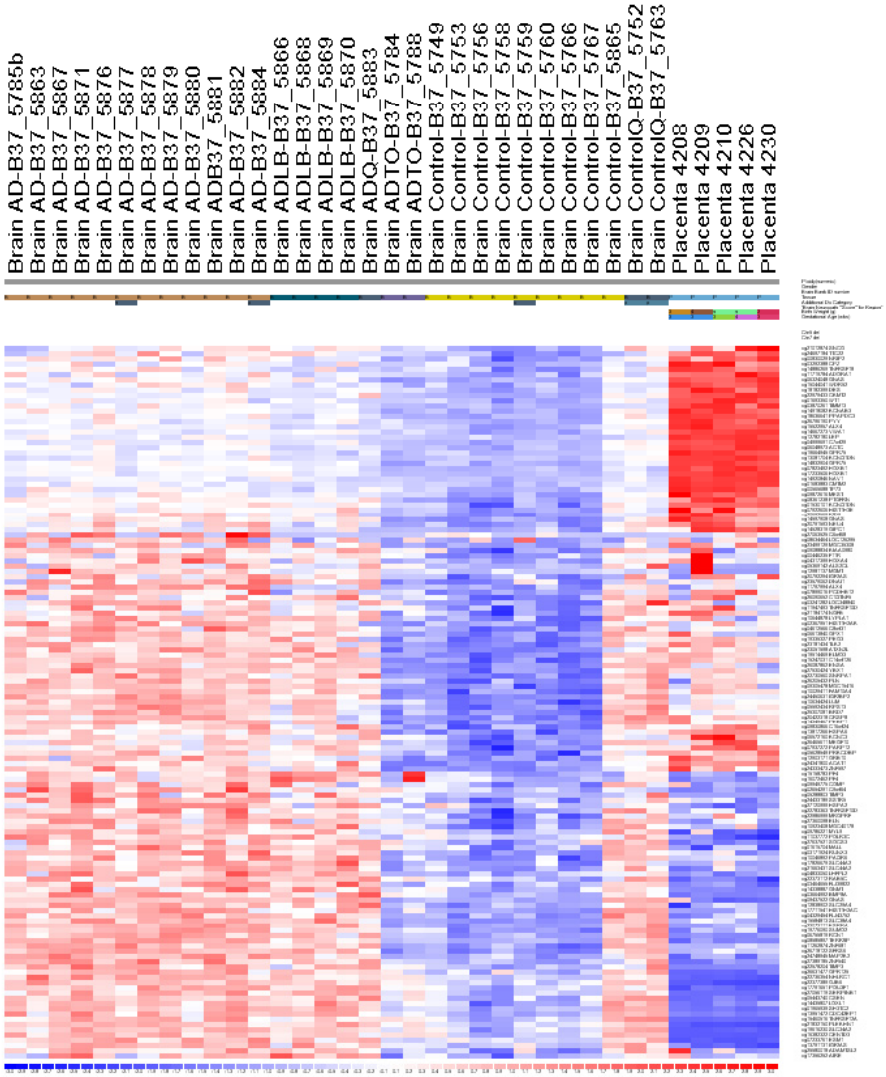


Y-axis: 2,300 genes with methylation fraction >.5 in at least 20% of the samples

Promoter meth: *few differences in AD vs. control*



Promoter meth: BA37 (temporal cortex) AD vs. control



Need to use lenient criteria to find anything:

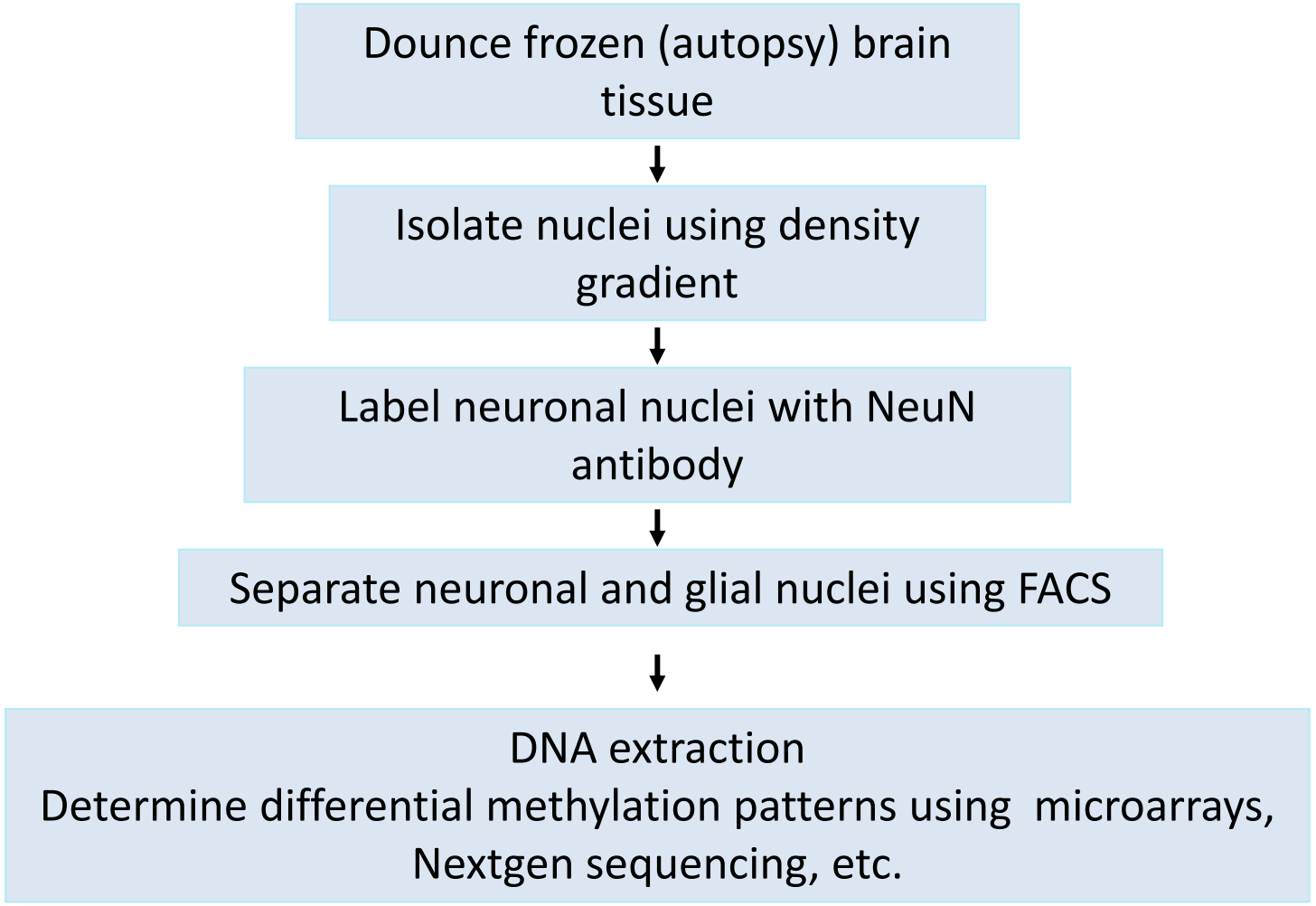
>10% absolute diff in methylation at a given gene;

p<.05 by simple ANOVA (not Bonferoni corrected)

AD vs. control whole temporal cortex: *hypermethylated promoter regions* associated with *decreased mRNA expression* in published data (AZ Sun Health group)

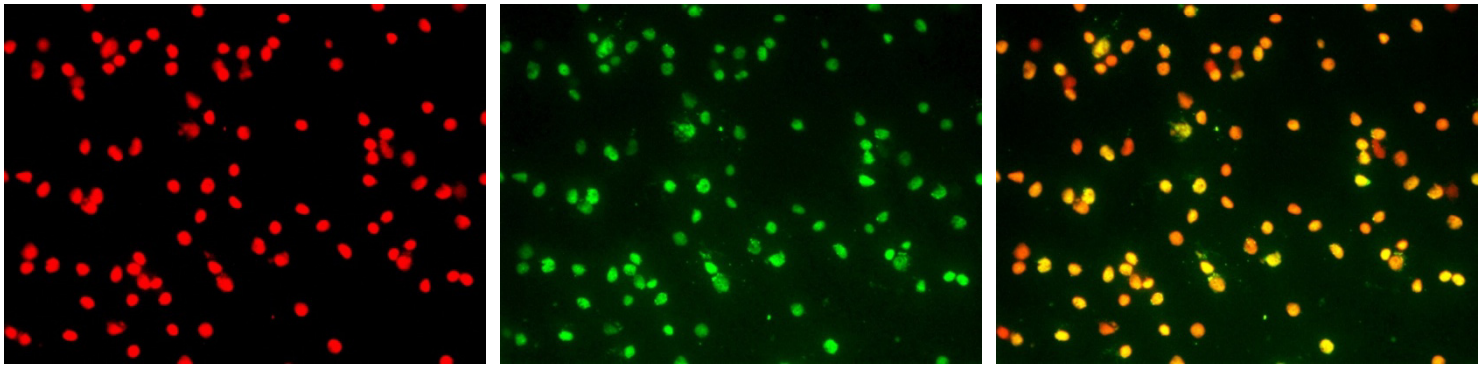
gene symbol	Brain regions	mRNA P-value	mRNA fold change AD vs. control	Methyl CpG fractional change AD vs. control
TERF2IP	HC	2.1E-06	-2.44	0.15
KCNC3	EC	7.9E-03	-3.34	0.12
SUMO3	HC	6.1E-03	-1.63	0.10
MGMT	MTC	1.5E-04	-2.24	0.10
C16orf24	EC	4.4E-04	-2.66	0.10
NPTX2	MTC	1.1E-05	-4.68	0.10

Separating neuronal vs. glial cells

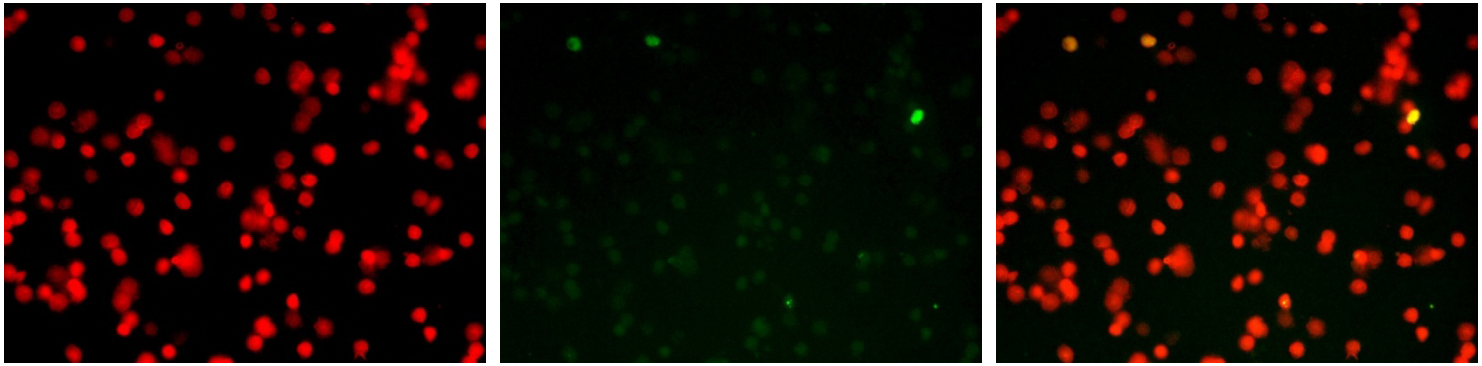


Sorted neurons from autopsy brains

NeuN+



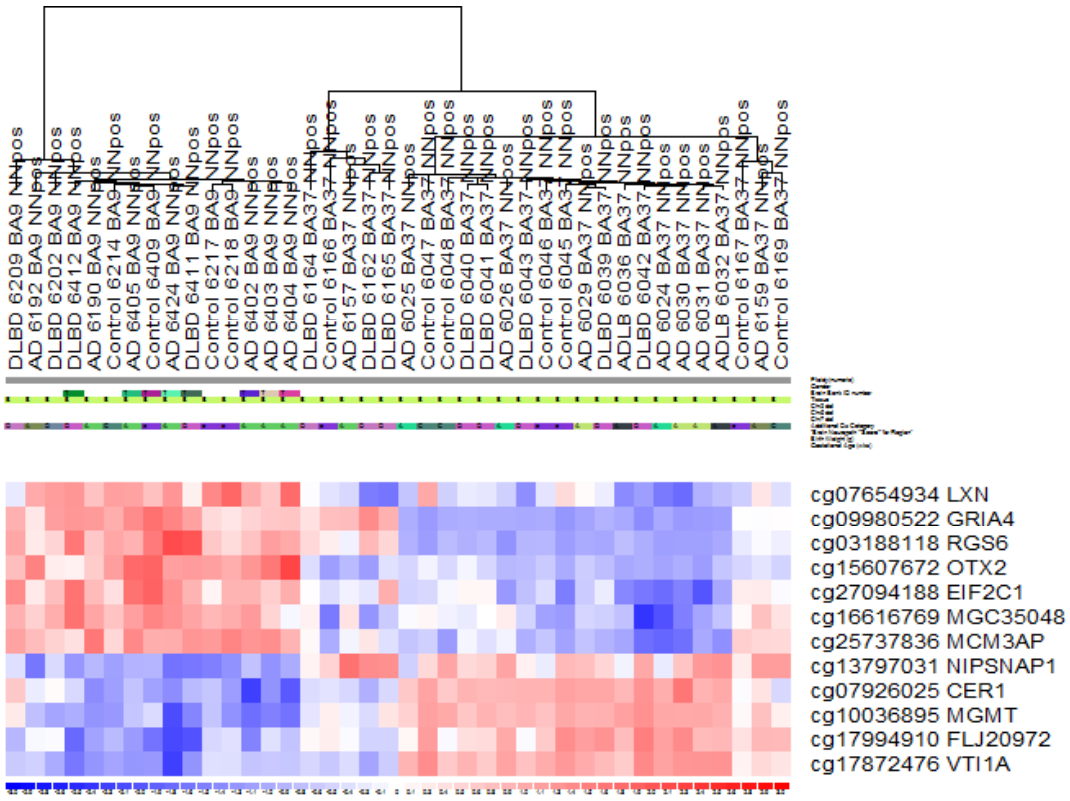
NeuN-



Sorted neurons: very few differentially methylated genes in AD vs. controls

- Infinium 27K “first generation” Beadarrays; mostly analyzing promoter regions; not other genomic regions.
- AD and control neurons from BA9 (frontal) and BA37 (temporal) cortex.
- Zero differentially methylated CpG’s in AD vs. control sorted neurons, even with lenient criteria.

…but there are clear differences in neuronal DNA methylation between different neocortical regions



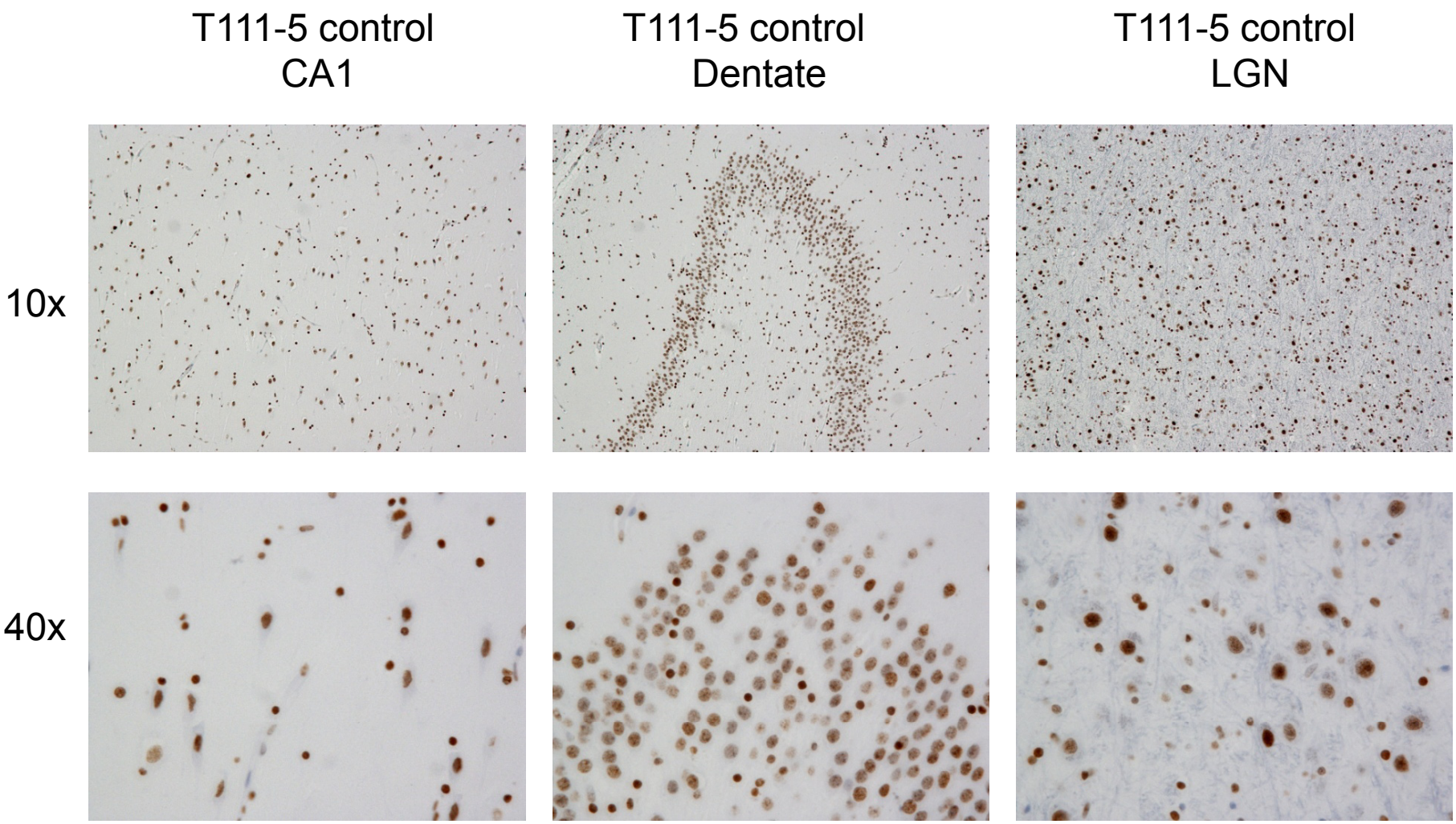
ANOVA p.05; require >15 % difference in methylation. BA9 control vs. BA37 control neurons. Compare samples on controls only; add AD and DLBD to the clustering.

Results from Infinium 27K

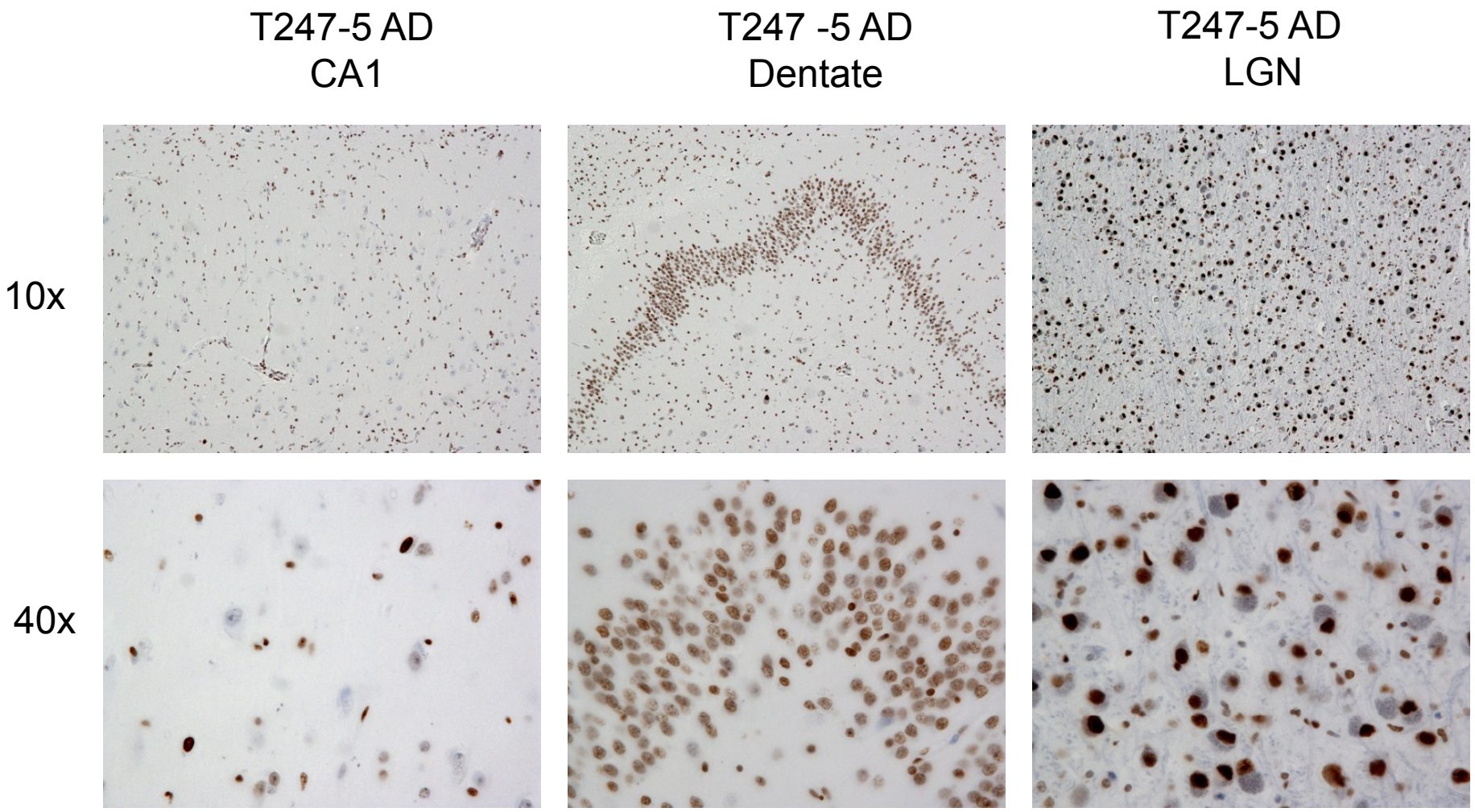
- In whole grey matter a few interesting genes do have mildly altered promoter CpG methylation in AD vs. control.
- In purified neurons we find almost no examples of genes with altered promoter methylation in AD vs. control.
- However, in purified neurons, promoter methylation varies substantially across different neocortical regions. Some of the differentially methylated genes are interesting.

Conclusion/hypothesis: patterns of promoter region DNA methylation likely help to **specify neuronal identities** in the human cerebral cortex but, except for a few interesting genes, these methylation patterns are **not strongly altered in the early to middle stages of AD.**

Assessing global methylation: anti-me-C



Globally reduced me-C in CA1 in AD?

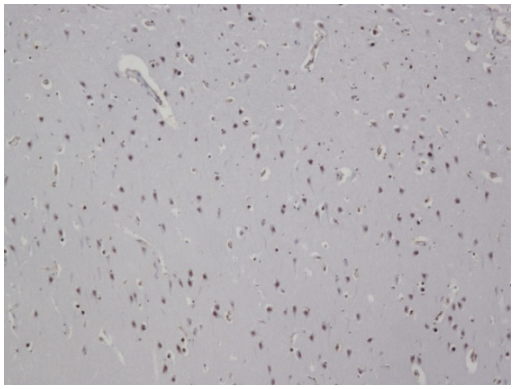
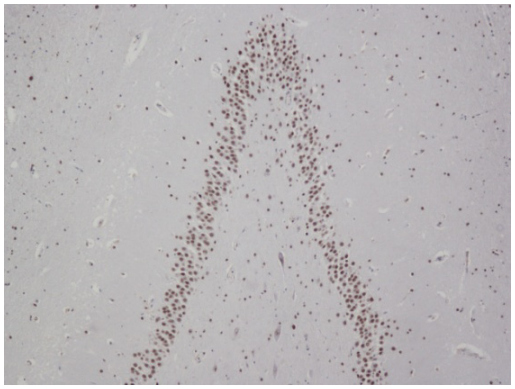
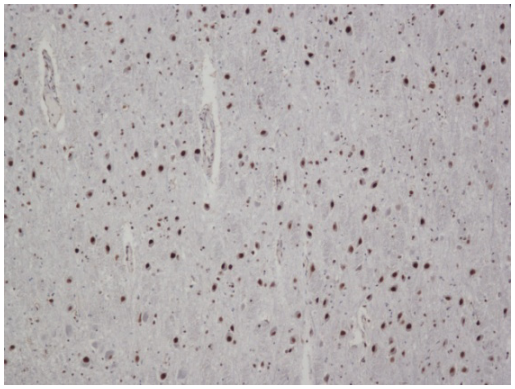


IHC using anti-OHmeC

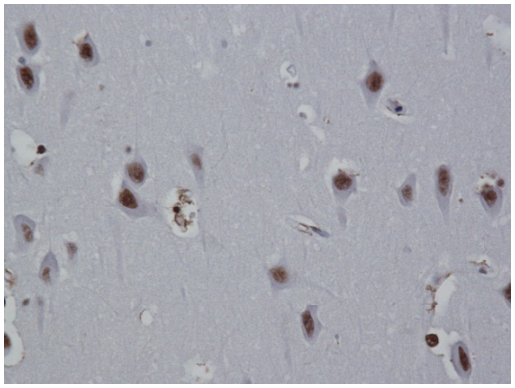
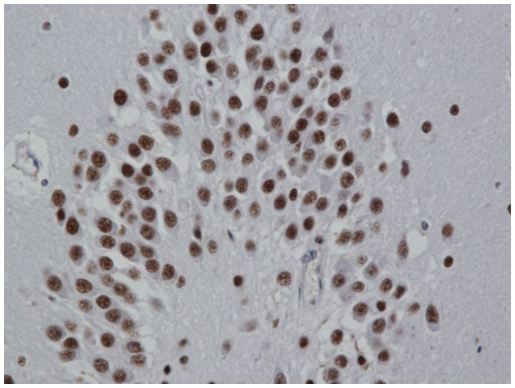
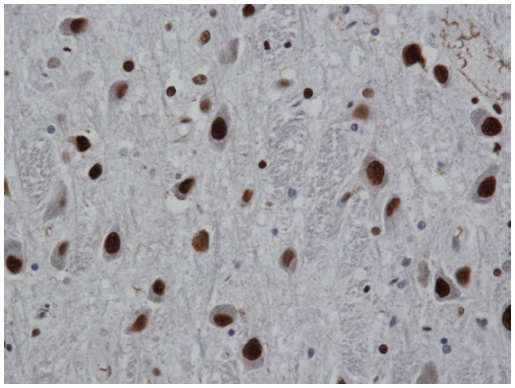
T99 Control

CA1 | Dentate | LGN

10x

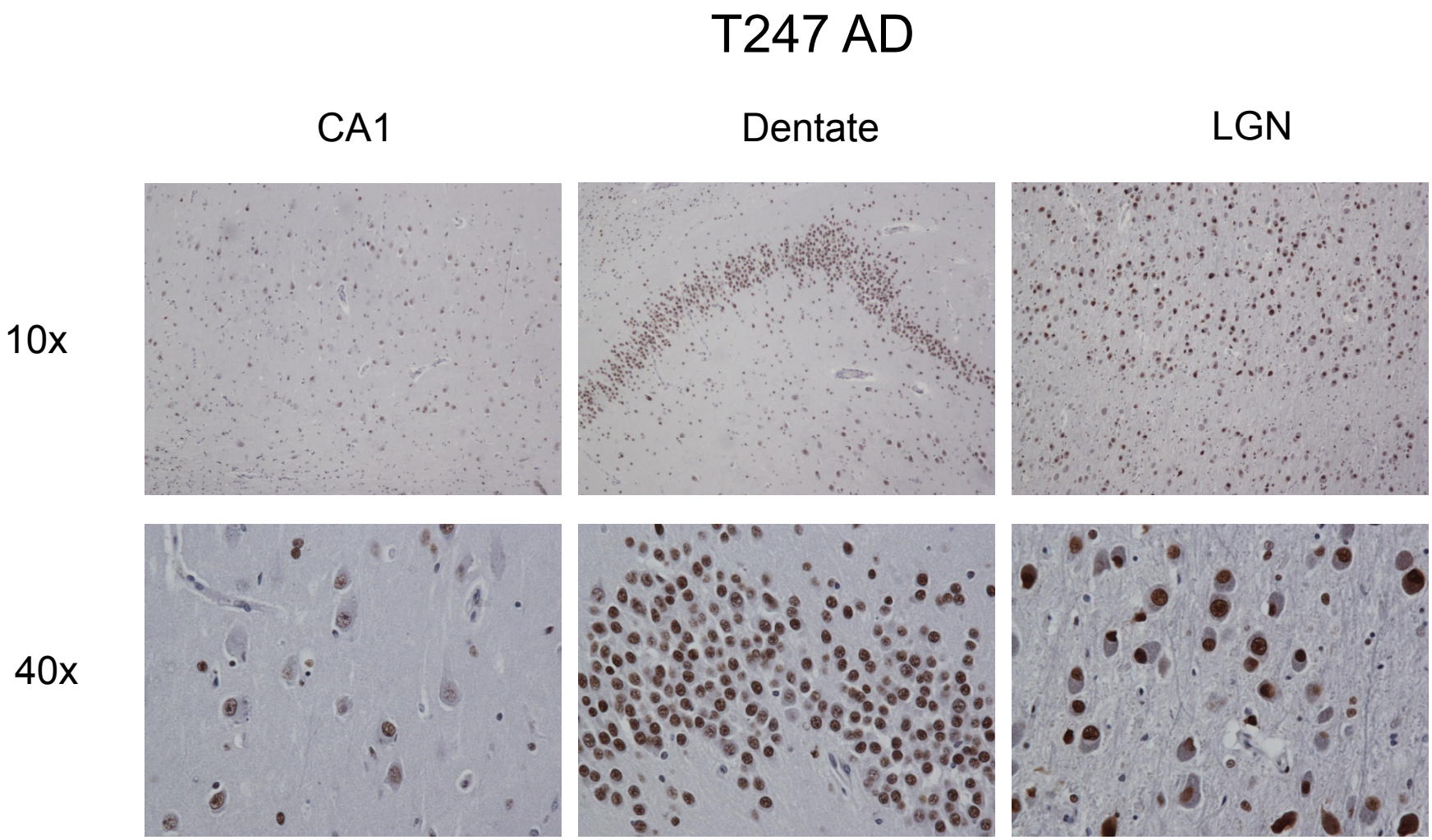




40x

Globally reduced OH-meC in CA1 in AD?

T247 AD



Global meC and OH-meC: work is still in progress

- Careful checking of technical issues (antigen accessibility) using other nuclear antigens
- Examining other brain regions in AD and control brains
- Careful correlations with neuropathology including plaques and tangles

DNA methylation and neuronal specification: what happens in Down syndrome?

- Methylation profiling and detailed studies comparing normal vs. Down syndrome cells
  - Blood cells (PBL, T-cells)
  - Cortical grey matter and purified neurons

Down Syndrome (trisomy 21)

Cognitive disability

Congenital heart defects

Early-onset Alzheimer's disease (1.5X APP)

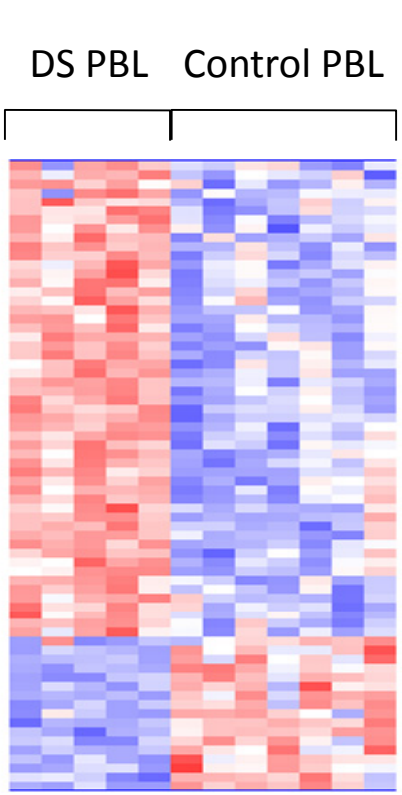
Childhood leukemias

Autoimmune diseases (50-fold increased)

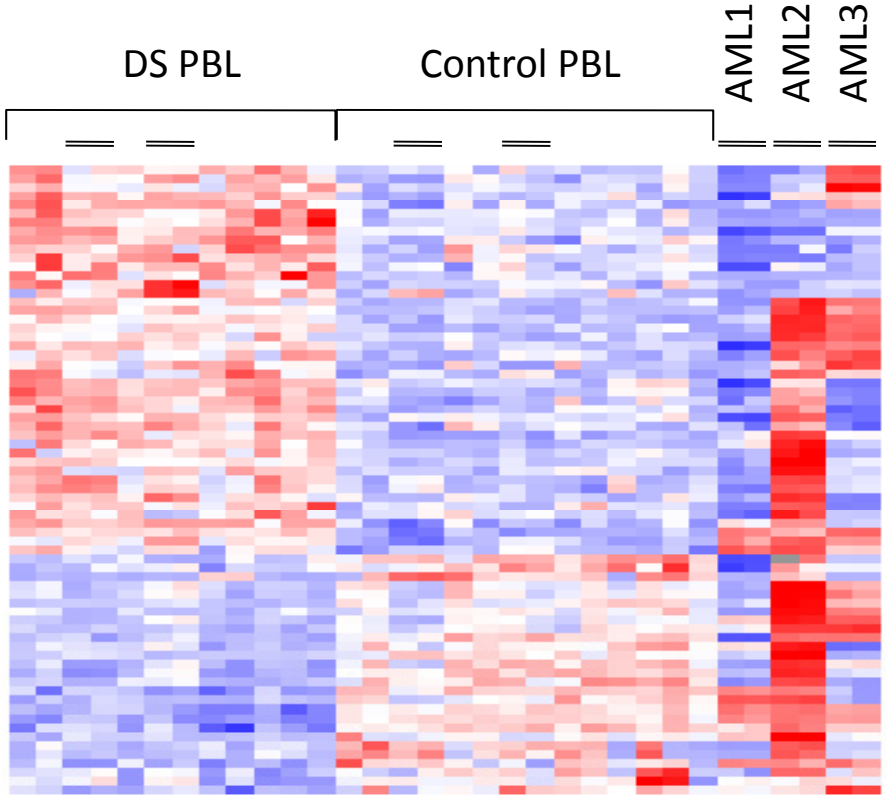
Recurrent infections

Altered lymphocyte and NK cell function

Altered patterns of CpG methylation in PBL from adults with DS

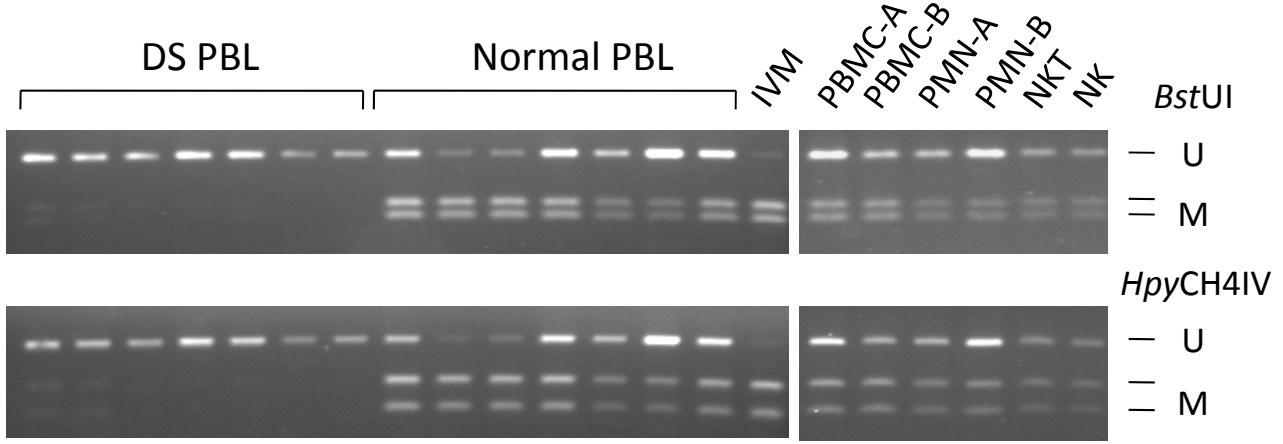


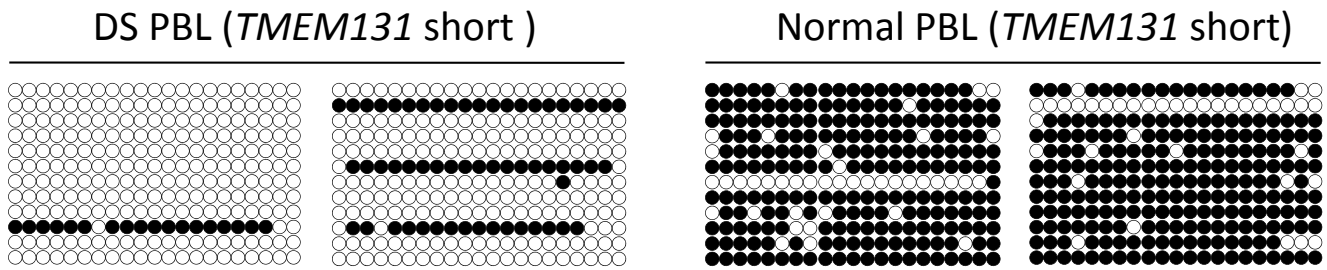
Affymetrix 250K MSNP

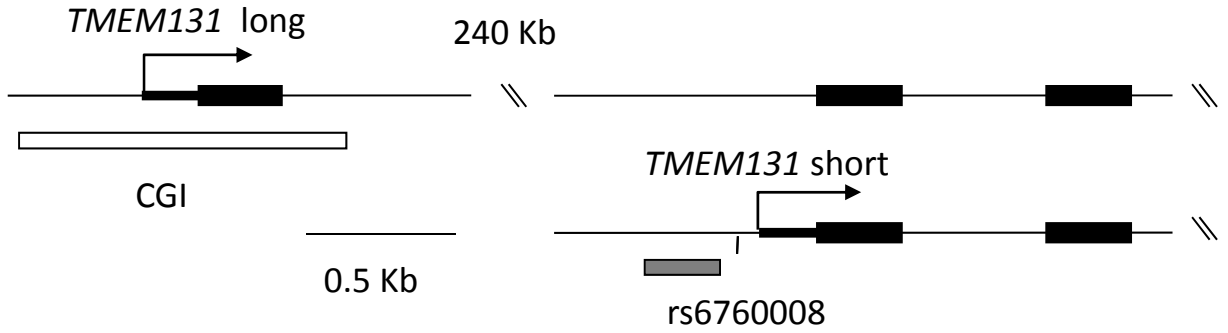


Illumina Infinium 27K

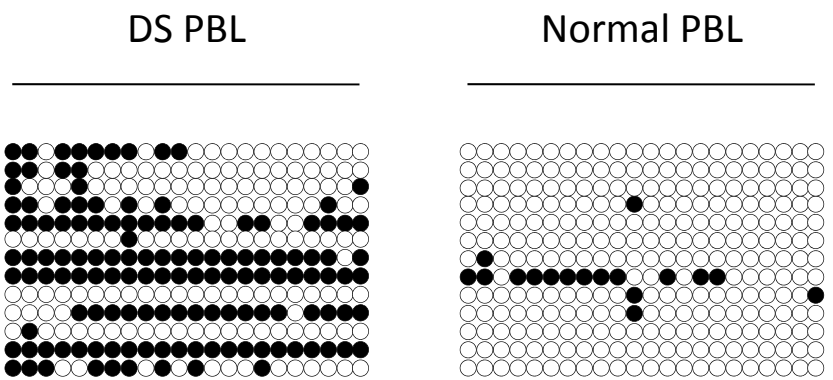
Validations: the *TMEM131* locus

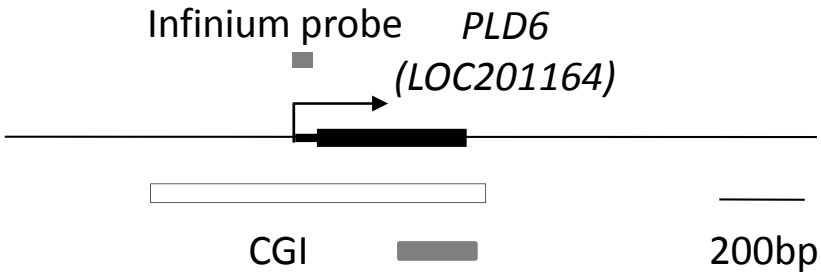


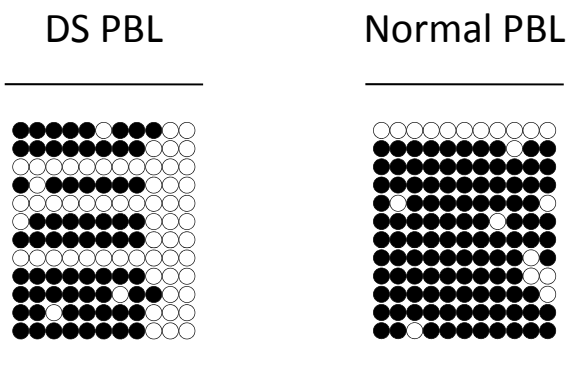


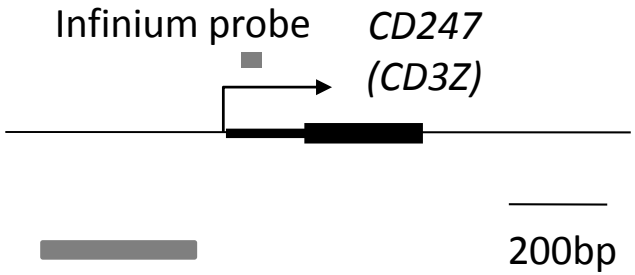


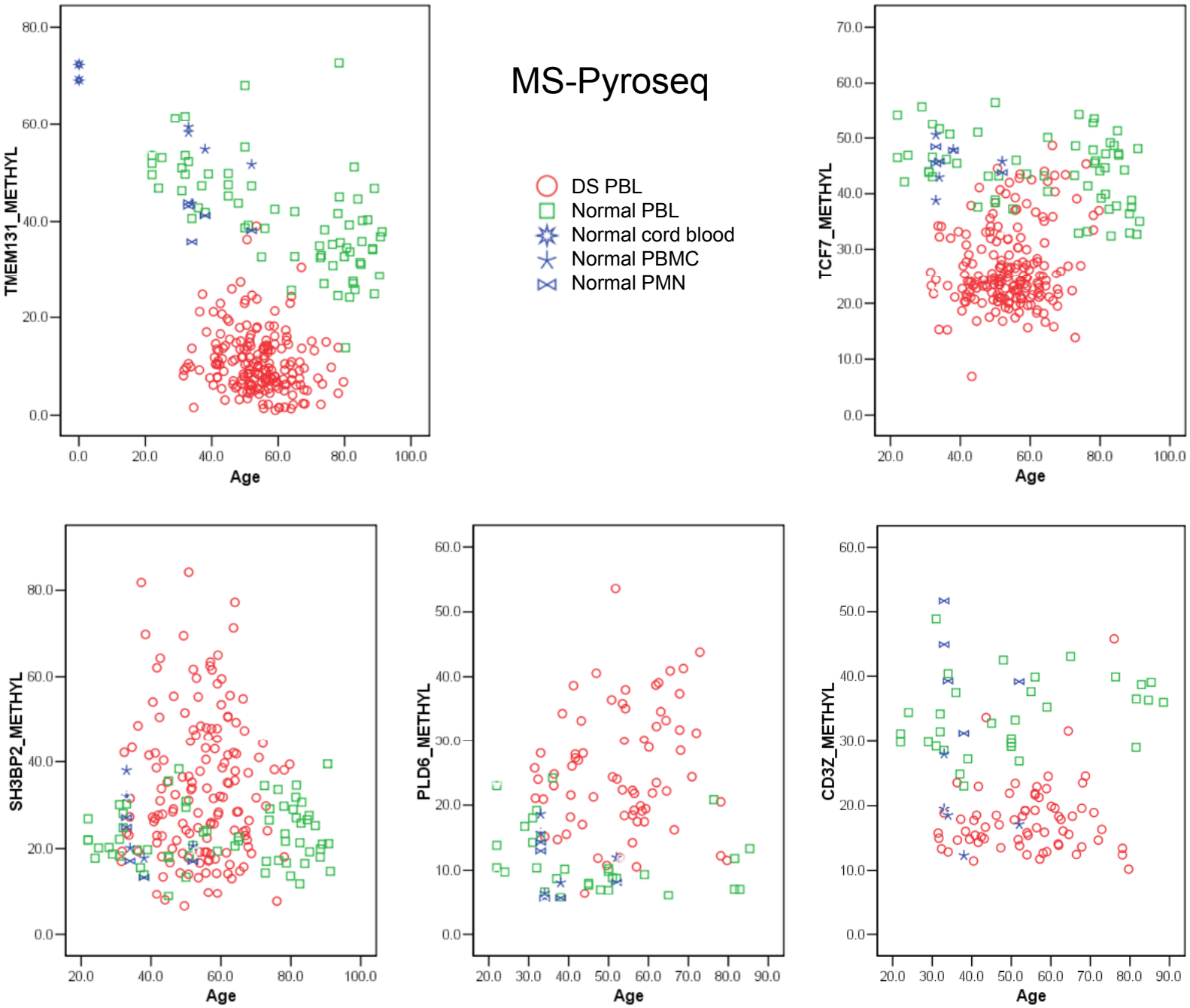
Validations: the *PLD6* and *CD3Z* genes



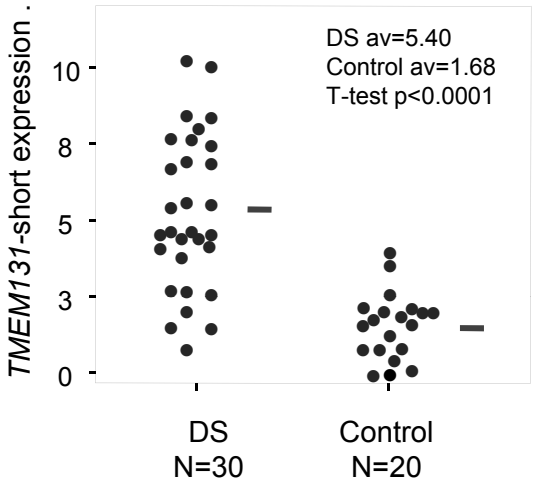


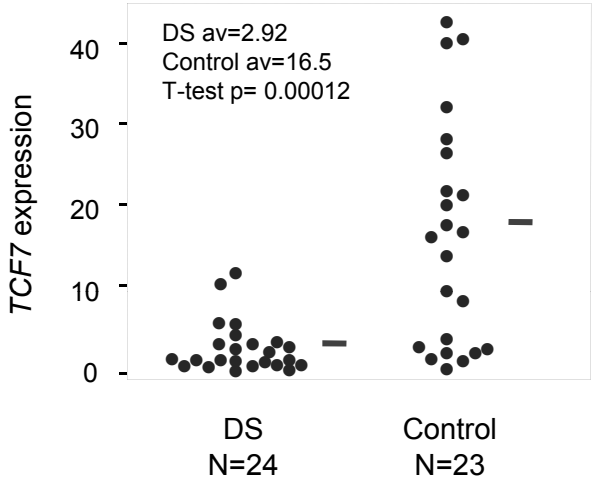


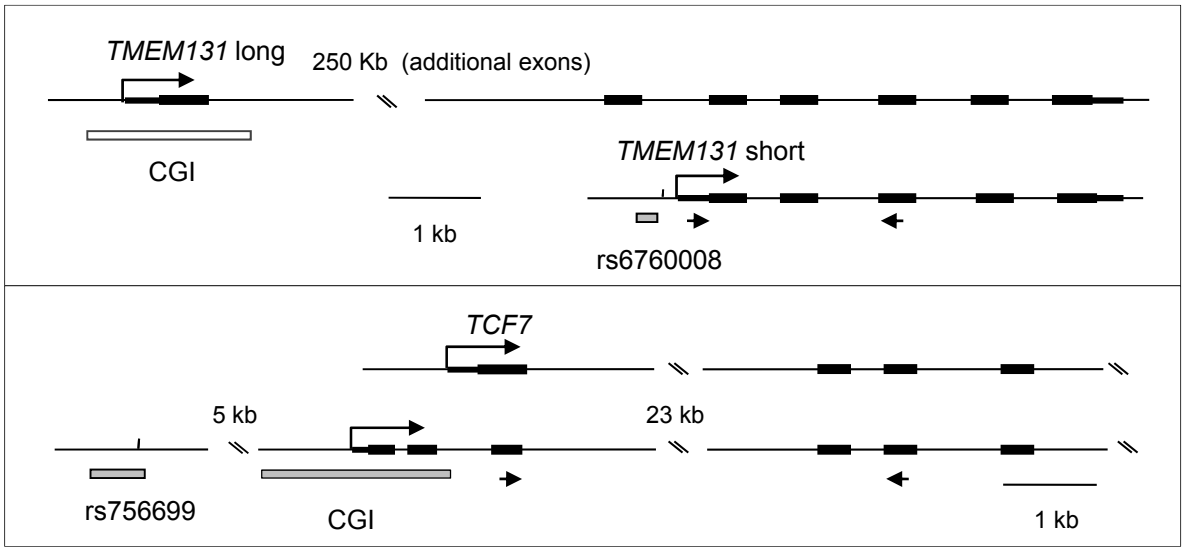



MS-Pyroseq
DS PBL
Normal PBL
Normal cord blood
Normal PBMC
Normal PMN
TMEM131_METHYL
TCF7_METHYL
SH3BP2_METHYL
PLD6_METHYL
CD3Z_METHYL
Age

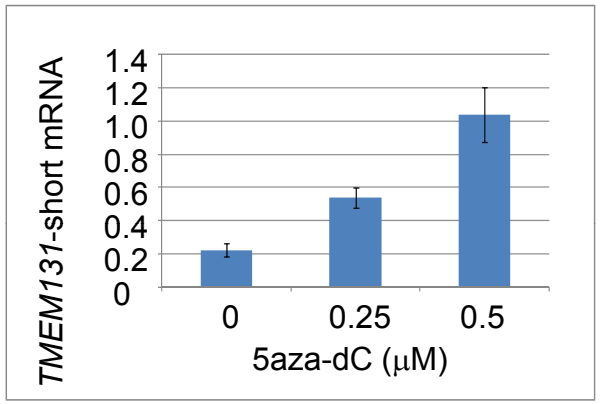
Altered mRNA expression of the differentially methylated genes in DS PBL



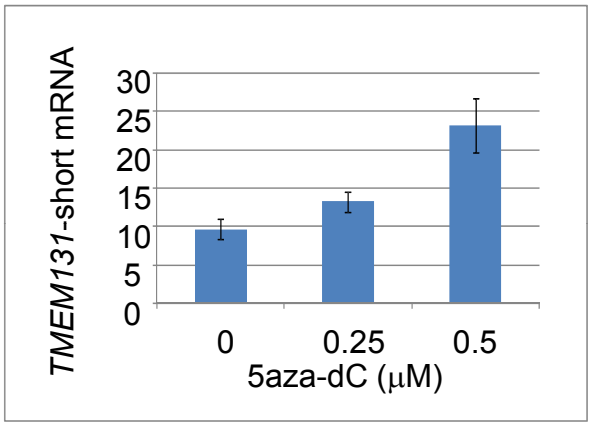




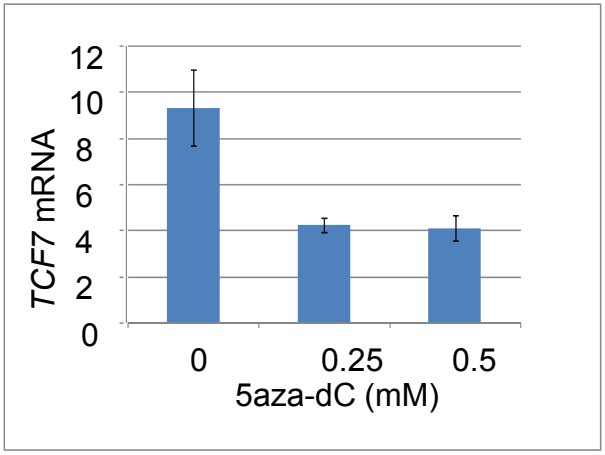
The altered methylation patterns are functionally relevant: gene activation



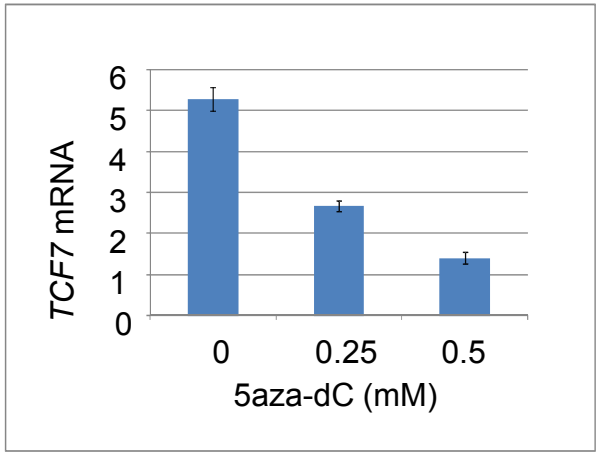
Jurkat cells



Primary PBMC, expanded with IL-15

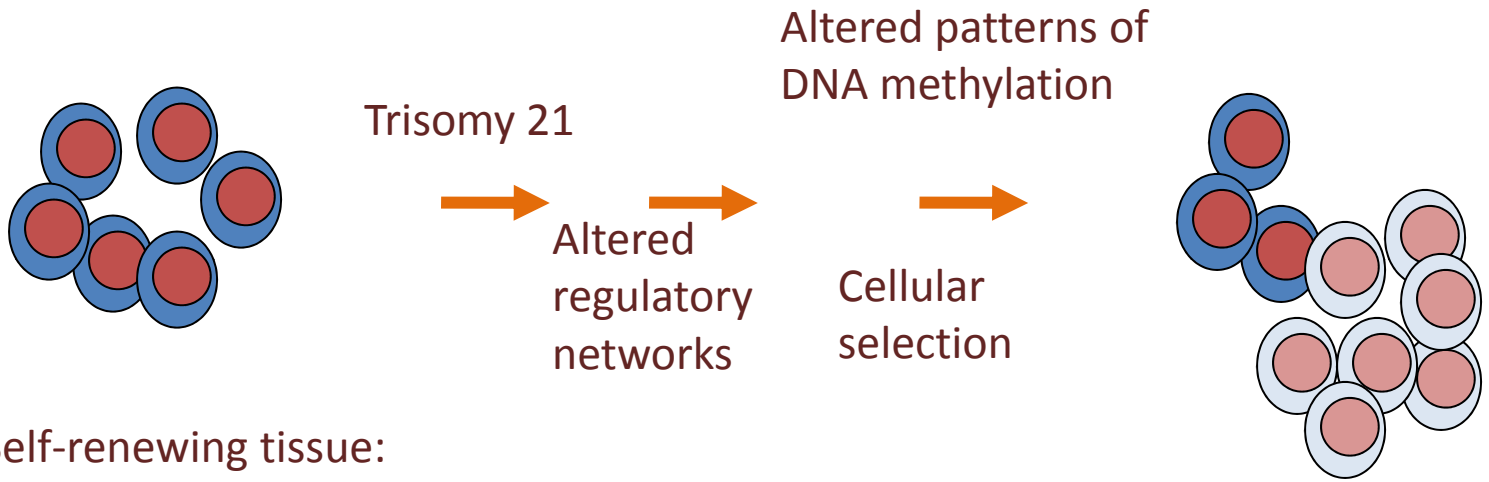


Jurkat cells



Primary PBMC, expanded with PHA

A programmed epigenetic response to a simple chromosomal aneuploidy in humans



Self-renewing tissue: hematopoietic system

Outgrowth of clones of cells with stereotypical epigenetic abnormalities. Genes are involved in lymphocyte function – candidates for producing **immune deficits and increased autoimmunity in Down syndrome and in the general population, i.e. Down syndrome as an "experiment of nature".**

PLoS One. 2010 Jun 28;5(6):e11357.

Genome-wide divergence of DNA methylation marks in cerebral and cerebellar cortices.

Xin Y, Chanrion B, Liu MM, Galfalvy H, Costa R, Ilievski B, Rosoklija G, Arango V, Dwork AJ, Mann JJ, Tycko B, Haghighi F.

Department of Psychiatry, Columbia University and The New York State Psychiatric Institute, New York, New York, United States of America.

Abstract

BACKGROUND: Emerging evidence suggests that DNA methylation plays an expansive role in the central nervous system (CNS). Large-scale whole genome DNA methylation profiling of the normal human brain offers tremendous potential in understanding the role of DNA methylation in brain development and function. METHODOLOGY/SIGNIFICANT FINDINGS: Using methylation-sensitive SNP chip analysis (MSNP), we performed whole genome DNA methylation profiling of the prefrontal, occipital, and temporal regions of cerebral cortex, as well as cerebellum. These data provide an unbiased representation of CpG sites comprising 377,509 CpG dinucleotides within both the genic and intergenic euchromatic region of the genome. Our large-scale genome DNA methylation profiling reveals that the prefrontal, occipital, and temporal regions of the cerebral cortex compared to cerebellum have markedly different DNA methylation signatures, with the cerebral cortex being hypermethylated and cerebellum being hypomethylated. Such differences were observed in distinct genomic regions, including genes involved in CNS function. The MSNP data were validated for a subset of these genes, by performing bisulfite cloning and sequencing and confirming that prefrontal, occipital, and temporal cortices are significantly more methylated as compared to the cerebellum. CONCLUSIONS: These findings are consistent with known developmental differences in nucleosome repeat lengths in cerebral and cerebellar cortices, with cerebrum exhibiting shorter repeat lengths than cerebellum. Our observed differences in DNA methylation profiles in these regions underscores the potential role of DNA methylation in chromatin structure and organization in CNS, reflecting functional specialization within cortical regions.

Recent publications from our group

PLoS Genet. 2010 Nov 18;6(11):e1001212.

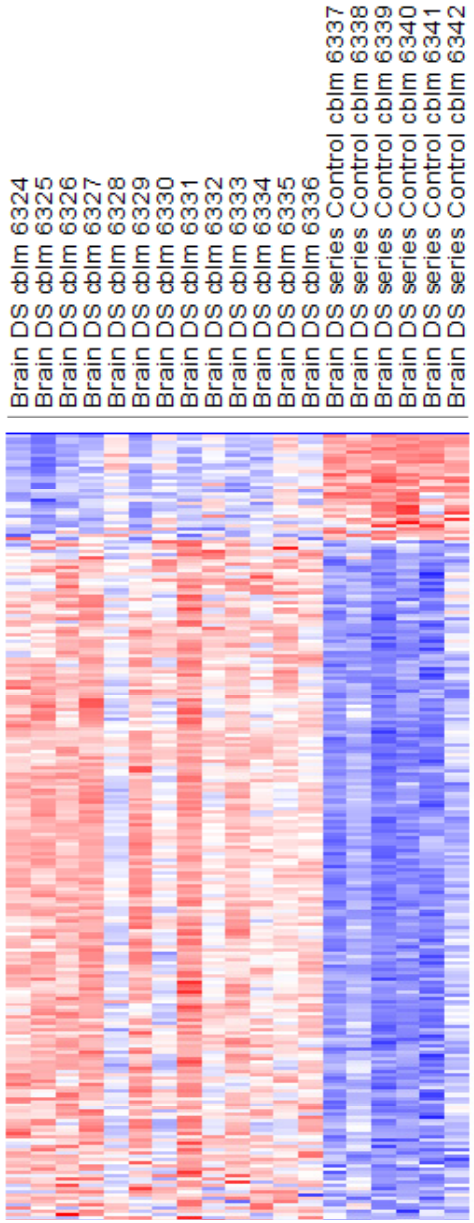
Altered DNA methylation in leukocytes with trisomy 21.

Kerkel K, Schupf N, Hatta K, Pang D, Salas M, Kratz A, Minden M, Murty V, Zigman WB, Mayeux RP, Jenkins EC, Torkamani A, Schork NJ, Silverman W, Croy BA, Tycko B.

Institute for Cancer Genetics, Columbia University Medical Center, New York, NY, USA.

Abstract

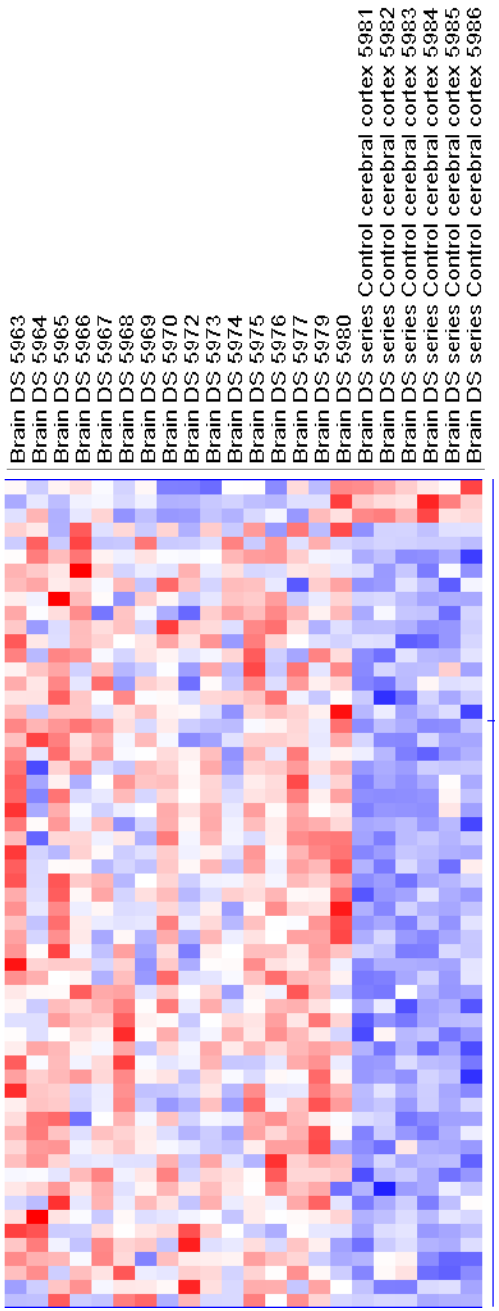
The primary abnormality in Down syndrome (DS), trisomy 21, is well known; but how this chromosomal gain produces the complex DS phenotype, including immune system defects, is not well understood. We profiled DNA methylation in total peripheral blood leukocytes (PBL) and T-lymphocytes from adults with DS and normal controls and found gene-specific abnormalities of CpG methylation in DS, with many of the differentially methylated genes having known or predicted roles in lymphocyte development and function. Validation of the microarray data by bisulfite sequencing and methylation-sensitive Pyrosequencing (MS-Pyroseq) confirmed strong differences in methylation ($p<0.0001$) for each of 8 genes tested: TMEM131, TCF7, CD3Z/CD247, SH3BP2, EIF4E, PLD6, SUMO3, and CPT1B, in DS versus control PBL. In addition, we validated differential methylation of NOD2/CARD15 by bisulfite sequencing in DS versus control T-cells. The differentially methylated genes were found on various autosomes, with no enrichment on chromosome 21. Differences in methylation were generally stable in a given individual, remained significant after adjusting for age, and were not due to altered cell counts. Some but not all of the differentially methylated genes showed different mean mRNA expression in DS versus control PBL; and the altered expression of 5 of these genes, TMEM131, TCF7, CD3Z, NOD2, and NPDC1, was recapitulated by exposing normal lymphocytes to the demethylating drug 5-aza-2'deoxycytidine (5aza-dC) plus mitogens. We conclude that altered gene-specific DNA methylation is a recurrent and functionally relevant downstream response to trisomy 21 in human cells.



Infinium 27K

Methylation DS vs normal cerebellum;

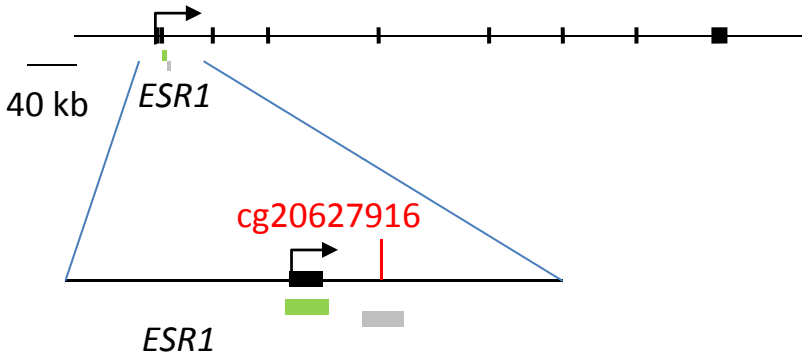
ANOVA p.05; 1.2fold diff; 0.1 absolute diff

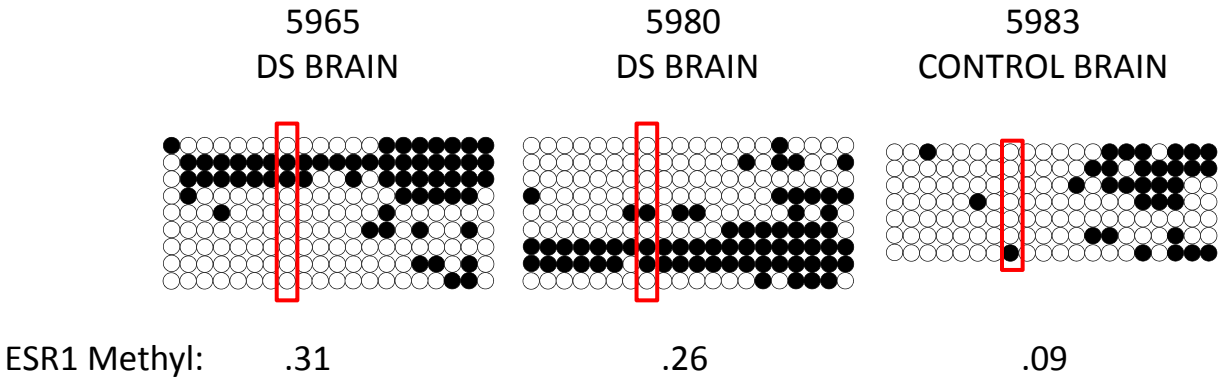


Infinium 27K

Methylation; DS vs control cerebral cortex; 1.2fold; 0.05diff; ANOVA p.05

ESR1: preliminary validations of differential methylation in DS vs. normal brains





ESR1 is a risk locus for AD in DS:

potential convergence of genetic and epigenetic data

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Estrogen receptor-alpha variants increase risk of Alzheimer's disease in women with Down syndrome.

Schupf N, Lee JH, Wei M, Pang D, Chace C, Cheng R, Zigman WB, Tycko B, Silverman W.

Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, G.H. Sergievsky Center, New York, NY, USA. ns24@columbia.edu

Abstract

BACKGROUND: Genetic variants that affect estrogen activity may influence the risk of Alzheimer's disease (AD). Two tightly linked polymorphisms (PvuII and XbaI) in the first intron of estrogen receptor 1 (ESR1), the gene for ER-alpha, have been reported to influence estrogen receptor expression and may influence the risk of AD.

METHODS: We examined the relation of polymorphisms in ESR1 to the risk of AD in women with Down syndrome. The subjects (181 women with DS, 41-78 years of age) were followed at 14- to 18-month intervals. Information from cognitive assessments, caregiver interviews, medical record reviews and neurological examinations was used to classify dementia. Genomic DNA was genotyped for 5 single-nucleotide polymorphisms in the upstream region and the first exon/intron of the ESR1 gene. Their association with dementia risk was evaluated, adjusting for covariates.

RESULTS: Women with at least 1 copy of the C allele at rs2234693 (PvuII) and those homozygous for the C allele at rs2077647 had an almost 3-fold increase in the risk of AD, compared with women without the C allele. The increased risks were independent of the apolipoprotein E genotype.

Current Research Group

- Huferesh Darbary
- Martha Salas
- Sandra Barral
- Maite Mendioroz
- Alexis Temkin
- Kristi Kerkel

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- Richard Mayeux
- Nicole Schupf
- John Crary
- J-P Vonsattel
- Jerzy Wegiel
- Wayne Silverman
- Warren Zigman
- Ed Jenkins