Epigenetic Approaches to Neurodevelopmental Disorders

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Plan for this talk

- Gedanken experiment: why epigenetics must be important in neurodevelopment
- CHARM: what is the nature of normal and abnormal DNA methylation in the genome?
- How does one apply high throughput epigenetics to the epidemiology of neurodevelopmental disorders?

Gedanken Experiment:

Why DNA methylation must be important

- Schroedinger's cat
- Maxwell's demon

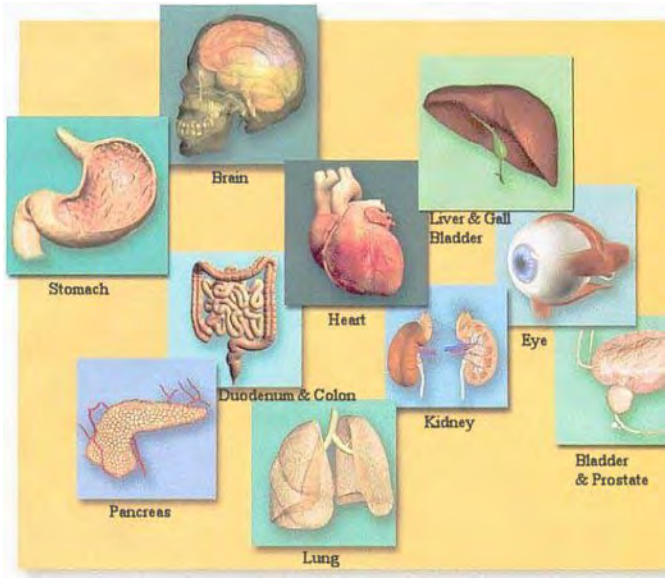
Gedanken Experiment

- Schroedinger's cat
- Maxwell's demon
- The United States Congress
- What makes them different?

3 billion base pairs of DNA
300,000 to 1,000,000 differences in DNA sequence

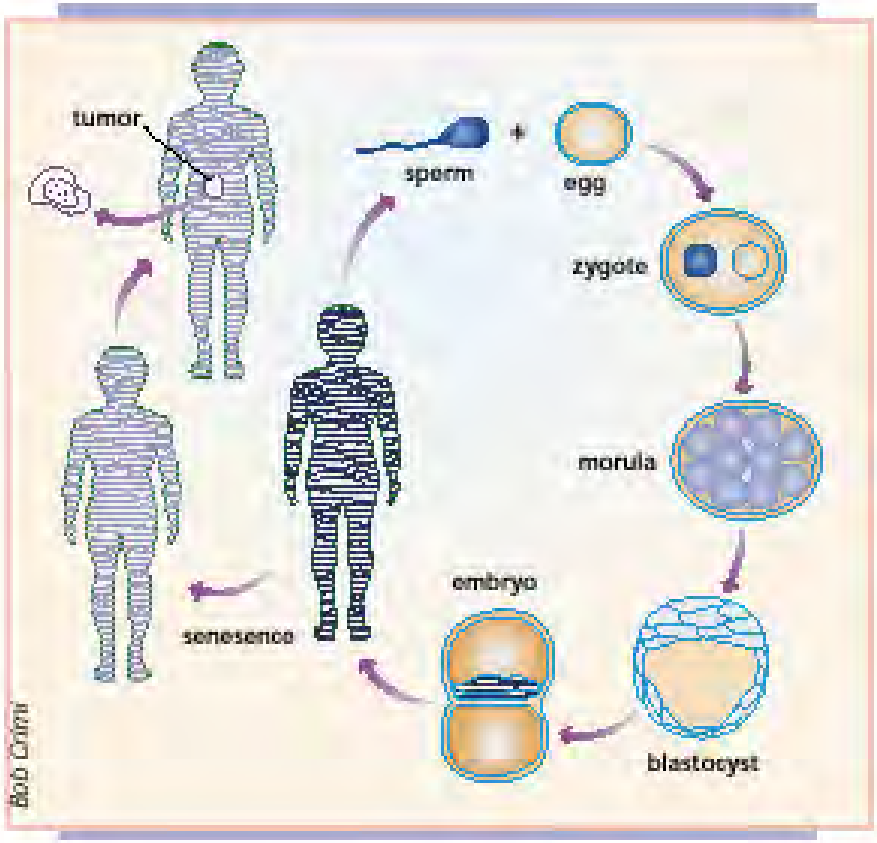
Gedanken Experiment

- Schroedinger's cat
- Maxwell's demon
- United States Congress
- Brain vs. heart vs. pancreas vs. eye



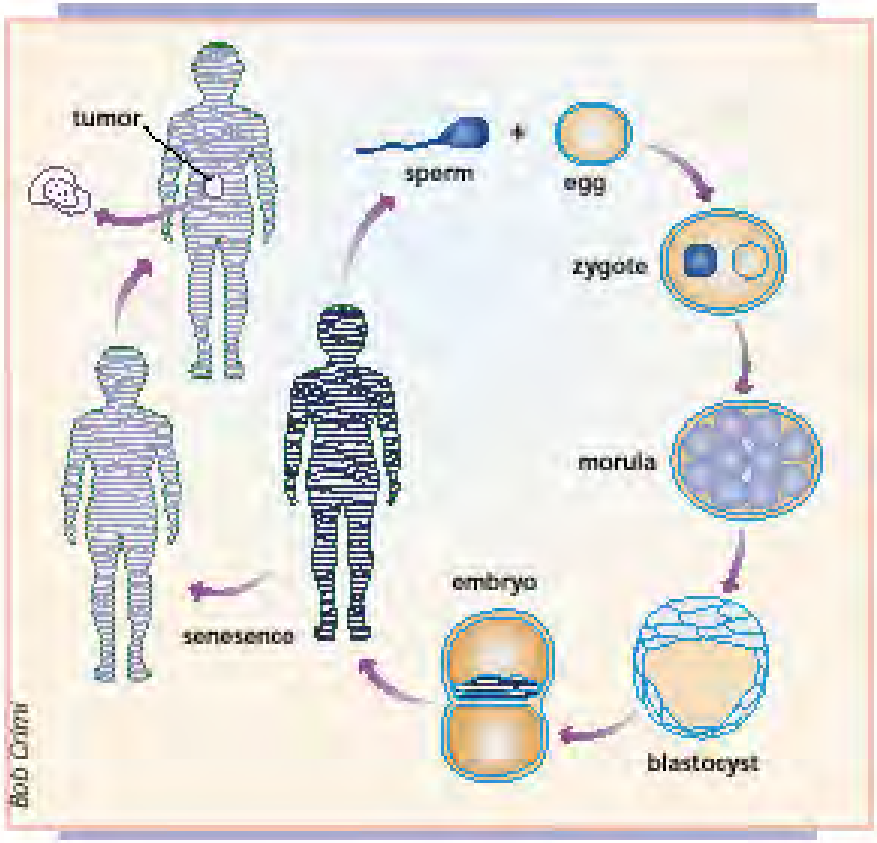
Far more different than the Congress
3 billion base pairs of DNA
0 differences in DNA sequence

Epigenetics has a life cycle, while the DNA sequence does not



Modifications of DNA or associated factors, that have information content and are maintained during cell division, other than the primary DNA sequence

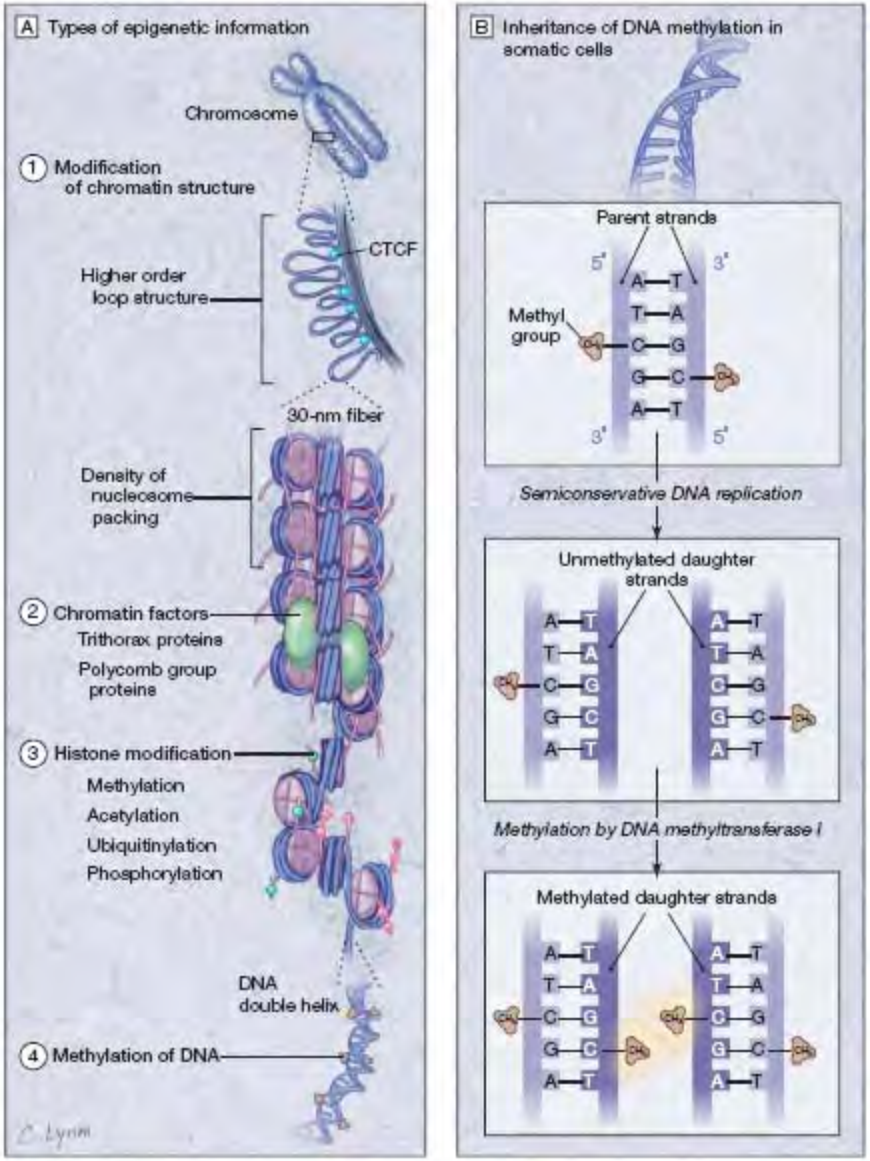
Epigenetics has a life cycle, while the DNA sequence does not



Epigenetic marks distinguish:

- Stem cells
- Tissue types
- Aging
- Cancer

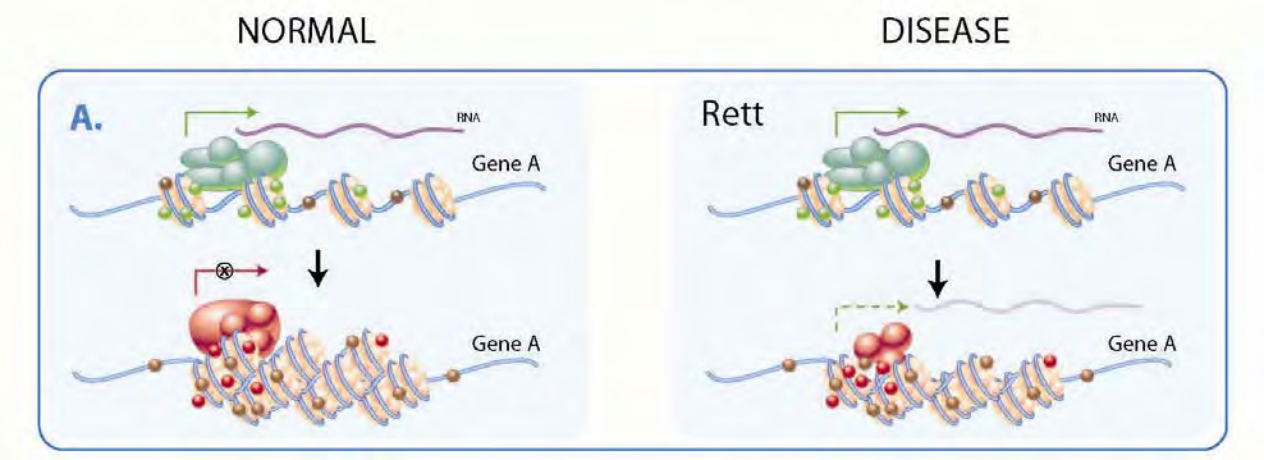
Types of epigenetic information



Disrupted epigenetics alters phenotypic plasticity

Rett syndrome = MeCP2 deficiency

Rett syndrome is a childhood neurodevelopmental disorder characterized by normal early development followed by loss of purposeful use of the hands, distinctive hand movements, slowed brain and head growth, gait abnormalities, seizures, and mental retardation. It affects females almost exclusively. --NINDS



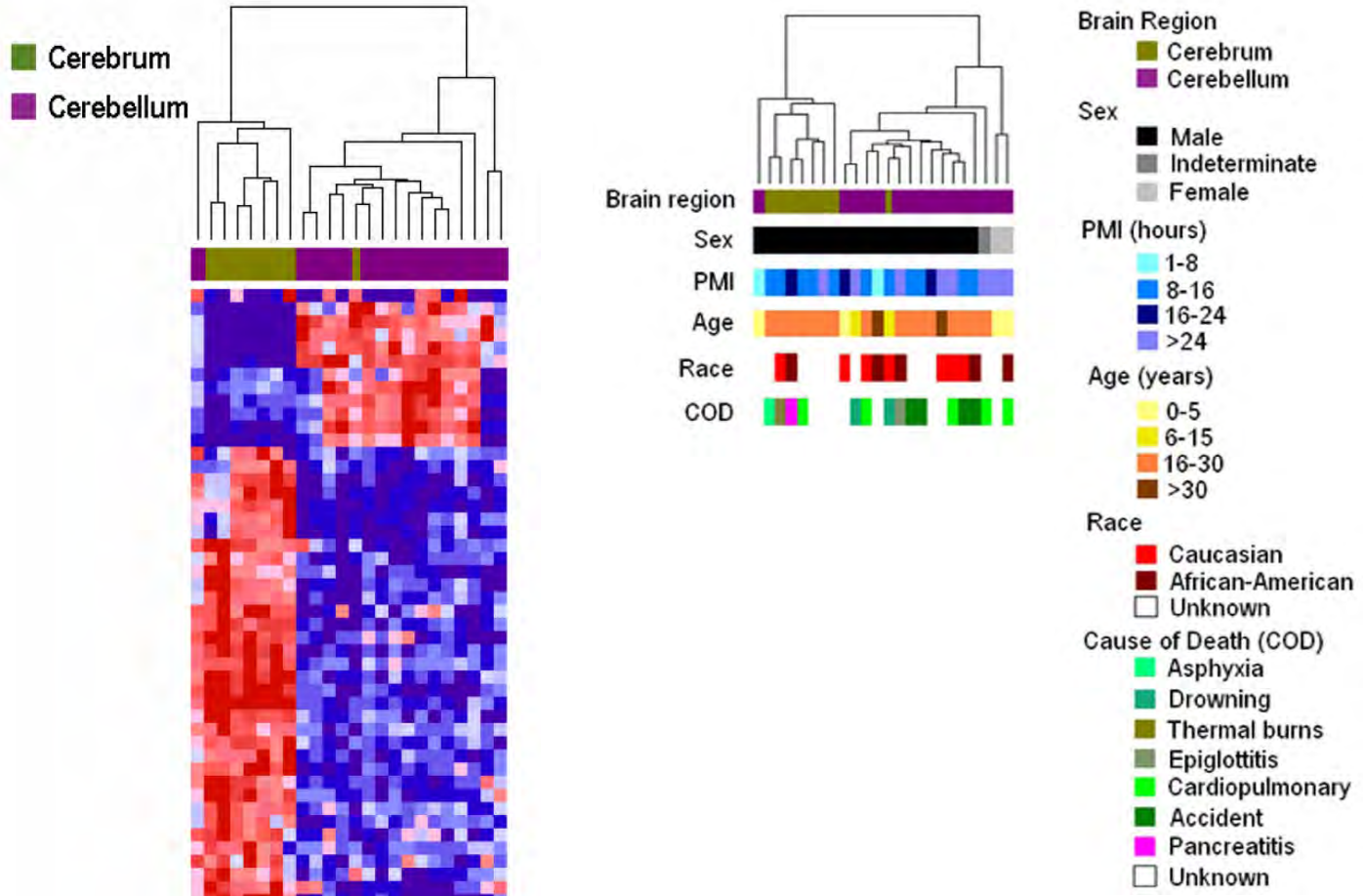
A failure to maintain and continue developmental modification (silencing)

Centrality of DNA Methylation

- Stable semi-permanent mark
  - Practical for human genetic studies
- Known mechanism for its propagation
  - DNA methyltransferase I is a Turing machine
  - No Turing machine yet for chromatin
- We need to assess DNA methylation genome-wide, cheaply, with high precision
  - Not with pre-existing methods
  - Previous assumption: "CpG islands" target of development and disease such as cancer
  - But is that true? Nobody ever checked.

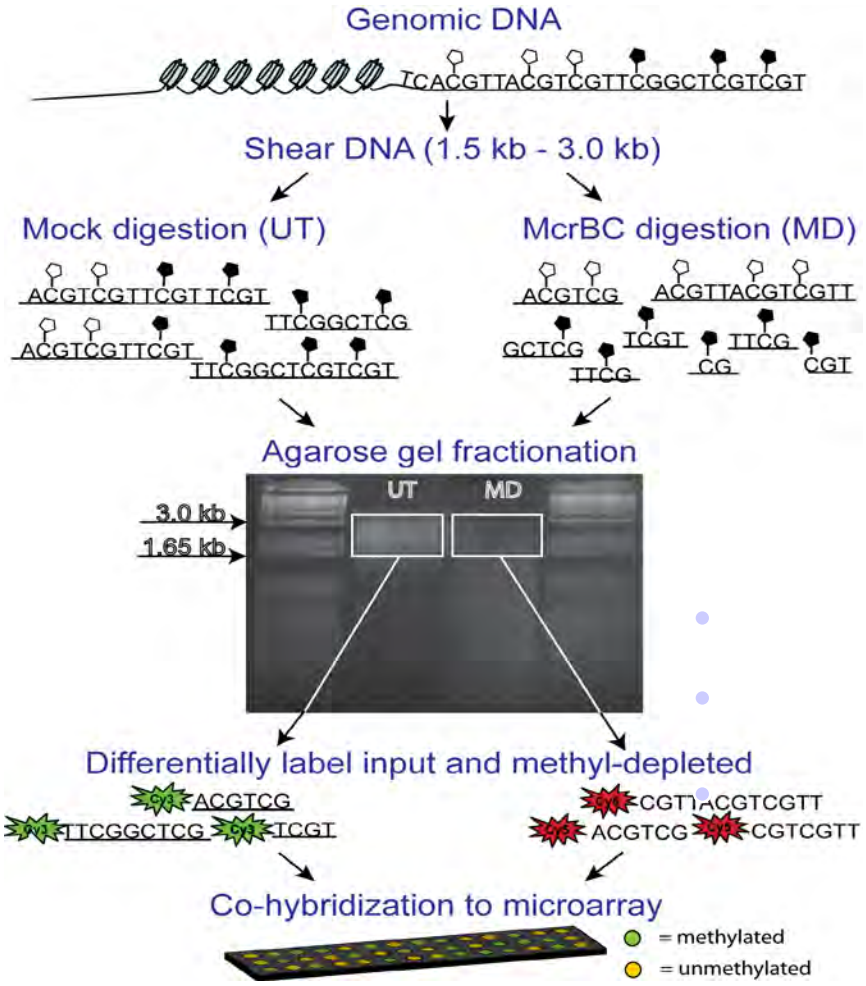
Does DNA methylation map the mind?

1500 "random" CpGs are as good as Ramon y Cajal



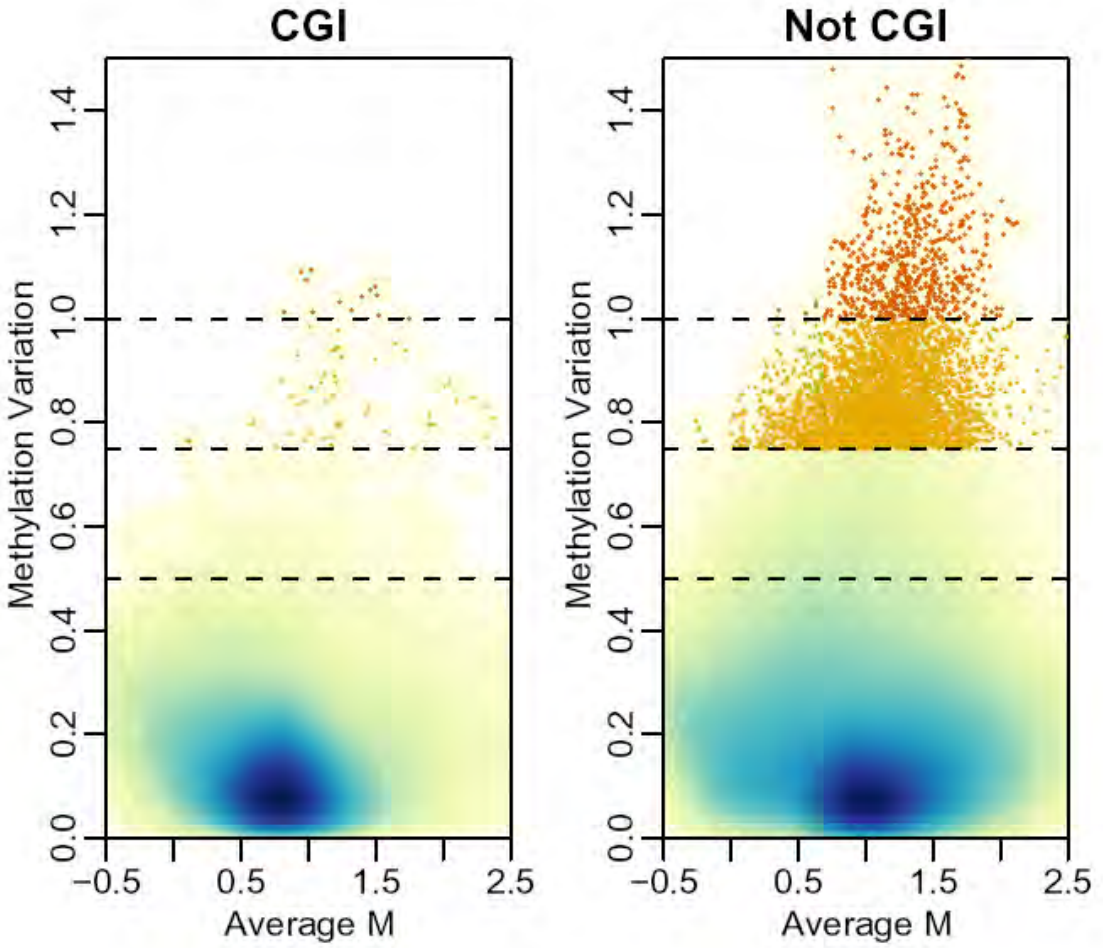
CHARM (Comprehensive high-throughput arrays for relative methylation)

What is the comprehensive map of normal and abnormal DNA methylation in the genome?

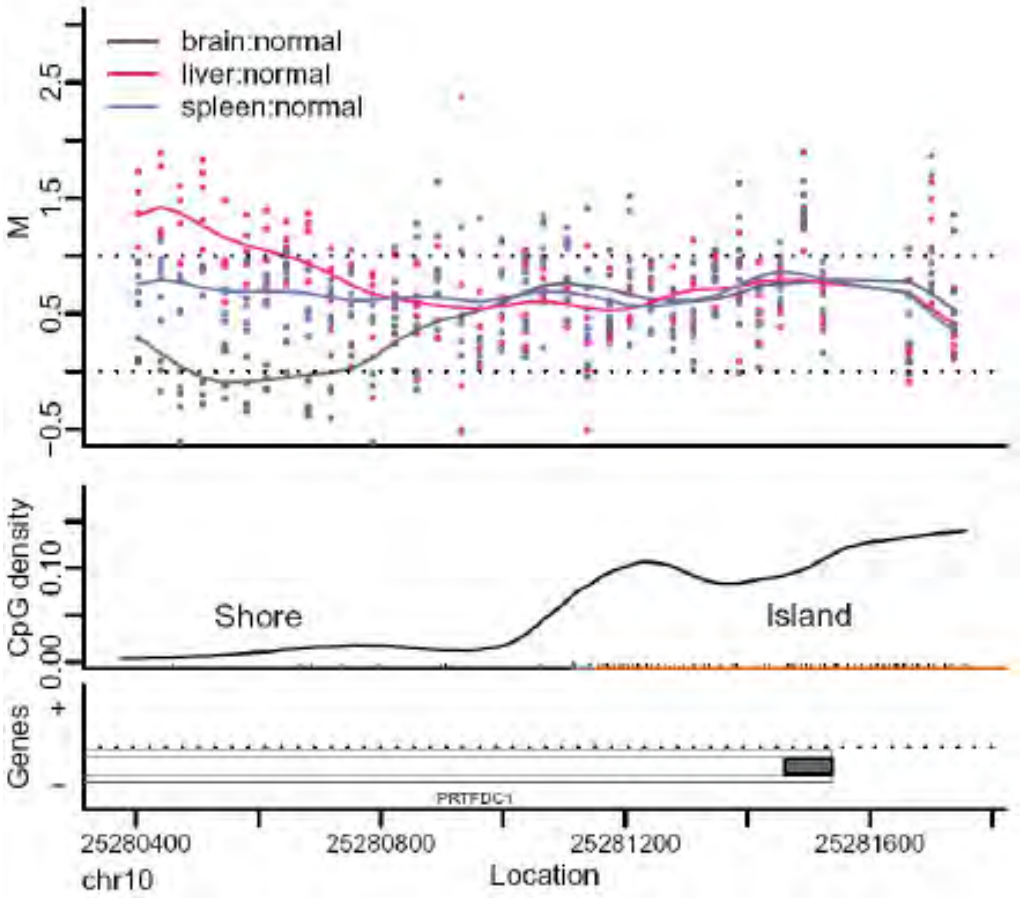


- ~ 4 million CpG sites measured
- Sensitive and specific
- Unbiased with respect to genomic region examined
- Includes all high-density "CpG islands" previously studied
- Also includes lower CpG regions not previously studied

Most methylation variation is outside CpG islands



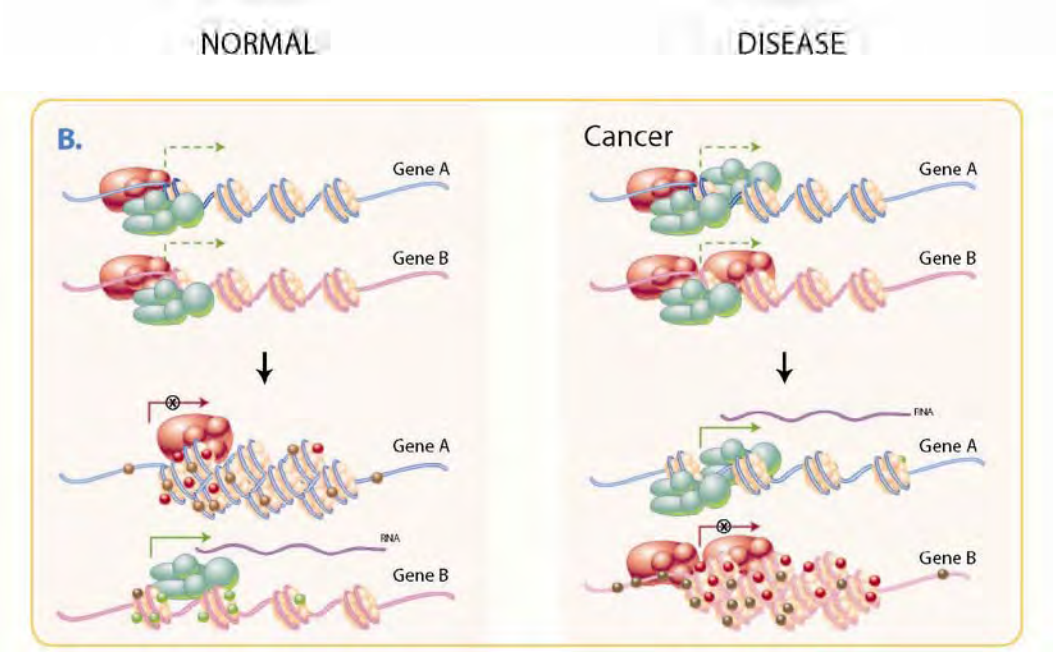
Where is normal DNA methylation?



- At CpG island "shores"

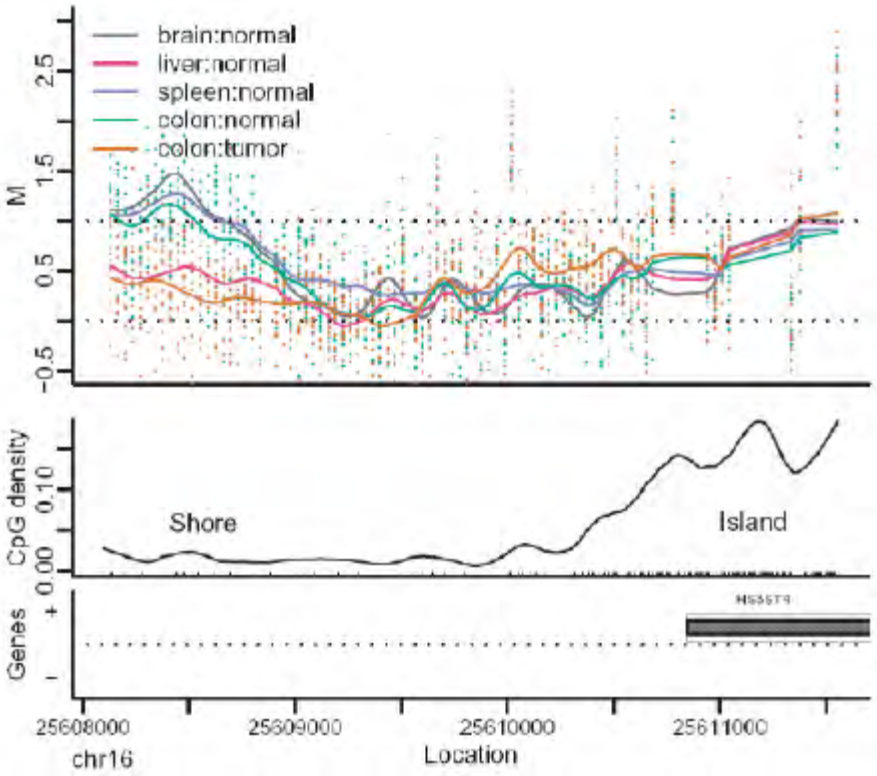
Epigenetic disease disrupts phenotypic plasticity

Complex trait: cancer



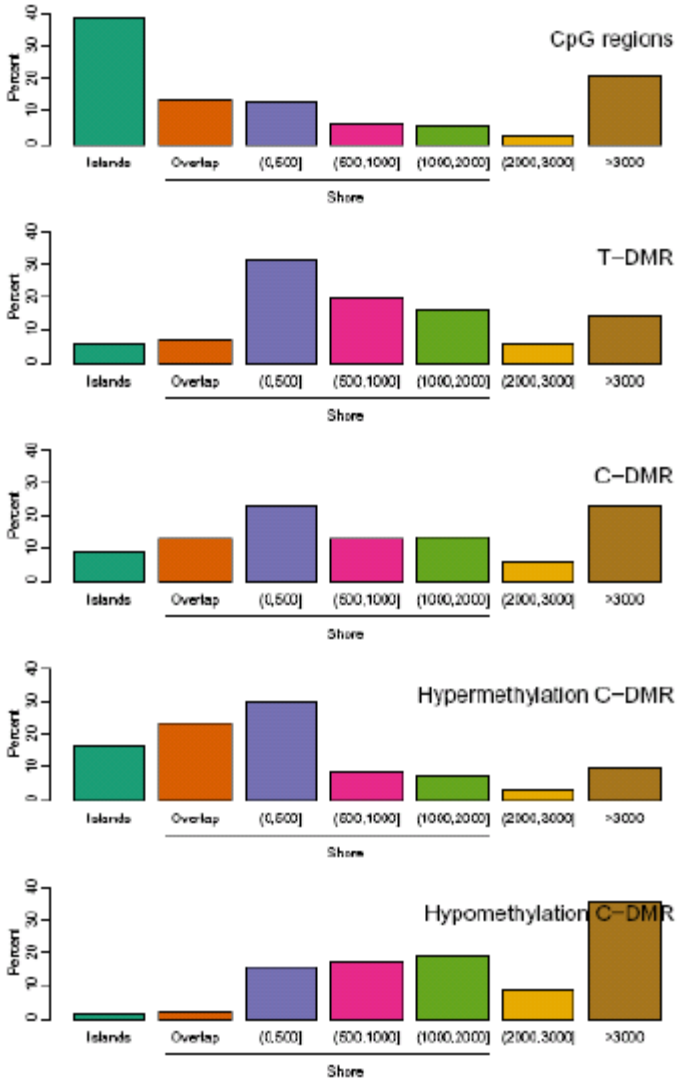
- Hypomethylation of many oncogenes
- Hypermethylation of many tumor suppressor genes
- Paradigm test of CHARM: Where is normal DNA methylation, and abnormal methylation in cancer?

Where is cancer DNA methylation?



- At CpG island shores, same ones as in tissues
- Acquires an aberrant pattern of tissue methylation

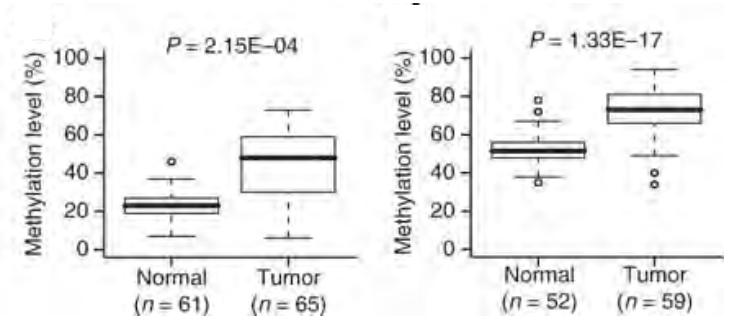
Relationship between C-DMRs and T-DMRs



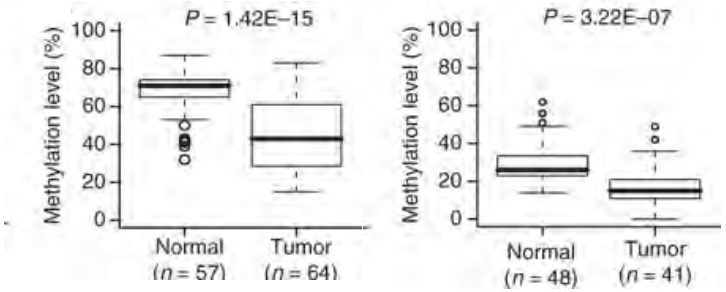
Validation of CpG island shores

Gene	Location[a]	Region	Tissue[b]	CG1	CG2	CG3	CG4	CG5	CG6	CG7	CG8	CG9	CG10	CG11	CG12	CG13	CG14	CG15	CG16	CG17	CG18
PCDH9	+3,338	Shore	Brain	32	26	12	39	19	22												
			Spleen	91	71	31	76	66	60												
			P value	<.001	<.001	<.001	<.001	<.001	0.003												
	-267	Island	Brain	2	3	4	2	3	5	2	3	2	3	3	3	5	5	4	3	3	4
			Spleen	2	3	3	2	3	6	2	4	3	3	3	3	3	5	4	3	4	3
			P value	0.032	0.298	0.336	0.108	0.475	0.150	0.393	0.141	0.011	0.661	0.265	0.208	0.420	0.051	0.133	0.885	0.783	0.270
HEY1	+3,381	Shore	Brain	54	53	51	51														
			Liver	70	84	87	71														
			P value	.023	<.001	<.001	<.007														
	+2,207	Island	Brain	4	7	3	4	4	5	1	8	5	5	7	7	4					
			Liver	3	6	3	4	4	6	2	9	23	26	26	8	8					
			P value	0.349	0.309	0.226	0.460	0.630	0.252	0.017	0.336	0.255	0.179	0.238	0.432	0.001					
HAGH	+2,192	Shore	Liver	26	30	22	18	7	6	23	33										
			Spleen	93	93	82	56	20	20	86	95										
			P value	<.001	<.001	<.001	<.001	<.001	<.001	0.017	0.017										
	+206	Island	Liver	2	1	2	3	2	7	3	2	3	1	2	2	1	1	1	2	1	4
			Spleen	2	2	3	4	2	2	2	2	4	1	4	2	2	3	2	4	1	8
			P value	0.608	0.207	0.433	0.803	0.058	0.342	0.262	0.529	0.504	0.782	0.060	0.832	0.366	0.074	0.307	0.073	0.141	0.015
SLMO2	+1,125	Shore	Normal	89	63	85	46	68	30	78	81	75	82	40	85	81	43	65	76	76	87
			Tumor	37	28	34	19	30	13	34	40	35	36	18	38	36	19	30	39	37	46
			P value	<.001	<.001	<.001	0.005	0.002	<.001	<.001	<.001	0.002	<.001	0.036	<.001	<.001	<.001	<.001	<.001	0.003	<.001
	+40	Island	Normal	4	2	3	3	6	4	3	2	3	2	3	3	4	2	7	5		
			Tumor	4	1	3	3	3	4	3	2	2	4	3	2	4	2	7	6		
			P value	0.619	0.233	0.293	0.546	0.302	0.364	0.461	0.204	0.586	0.263	0.173	0.369	0.253	0.928	0.230	0.509		

Bisulfite pyrosequencing validation in >50 tumor-normal pairs

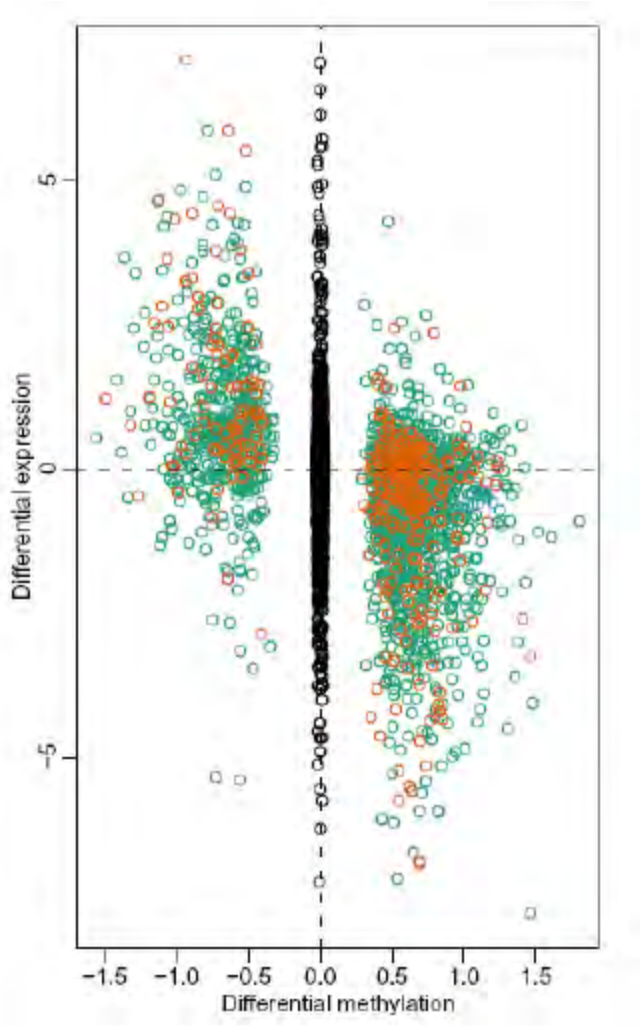


hypermethylation

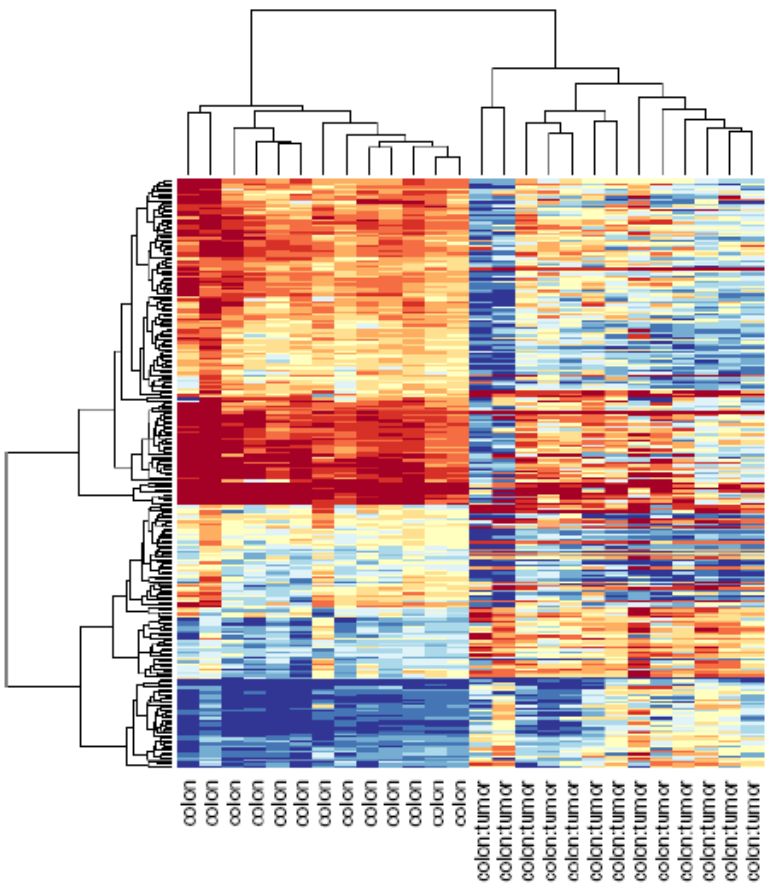


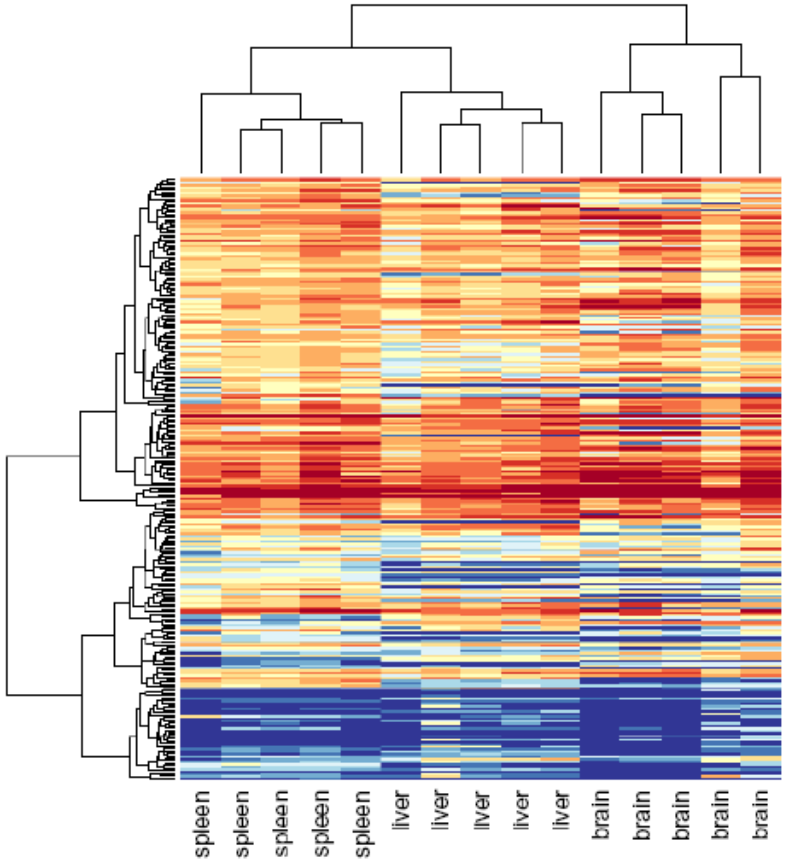
hypomethylation

Methylation of DMRs functional



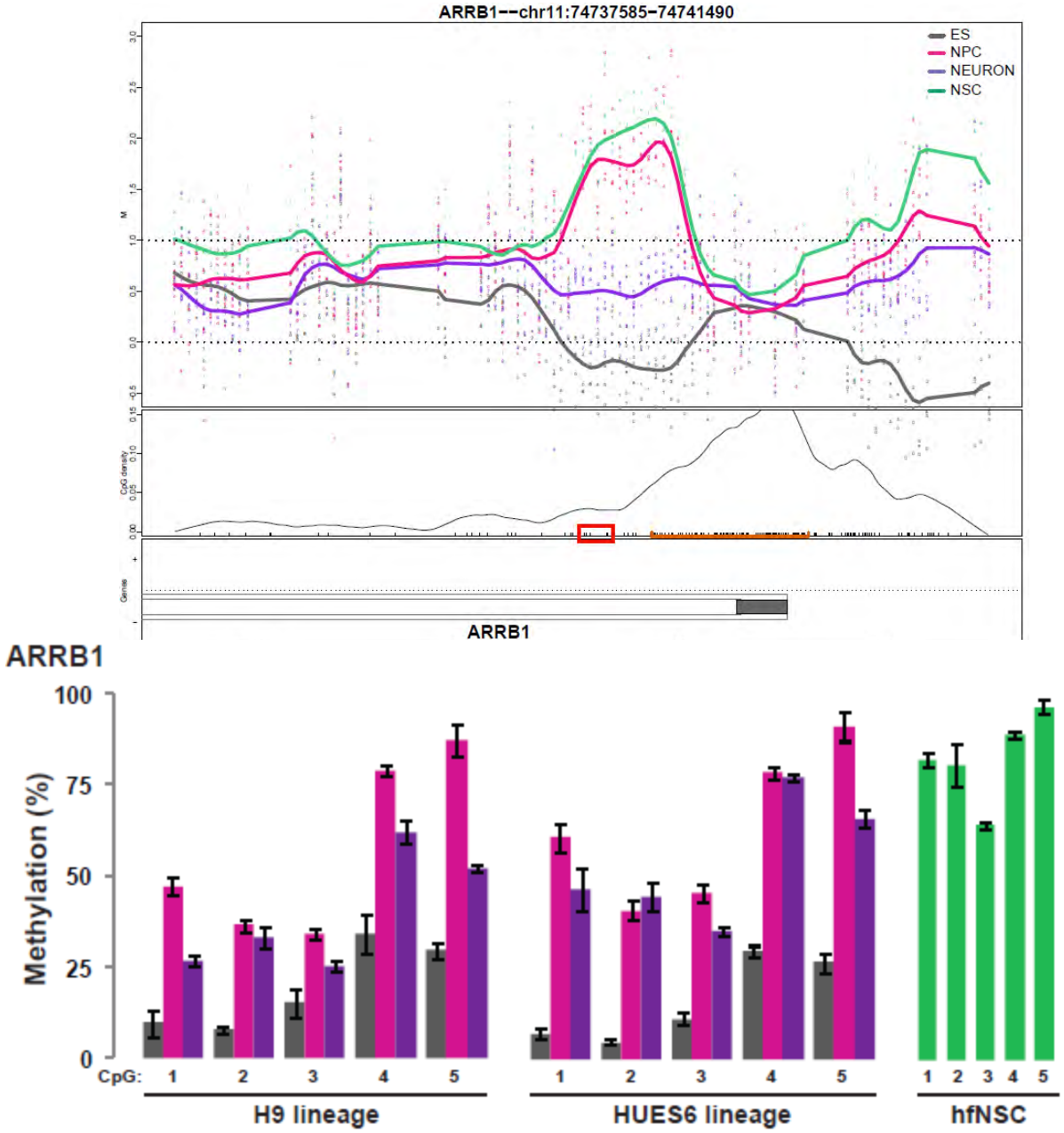
Cancer methylation predicts tissue methylation



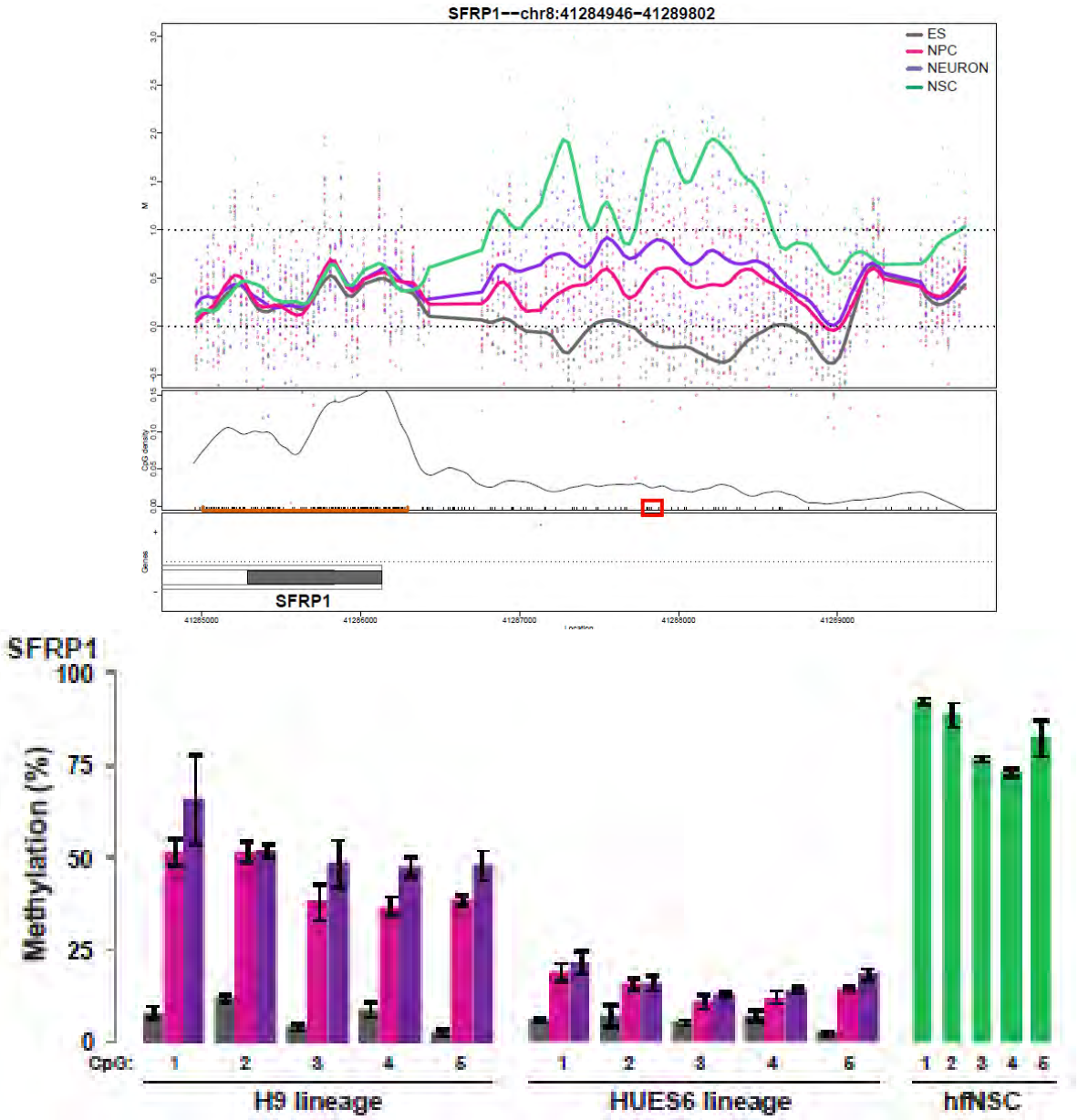


Further evidence that normal differentiation

Same DMRs involved in neural differentiation



Same DMRs involved in neural differentiation



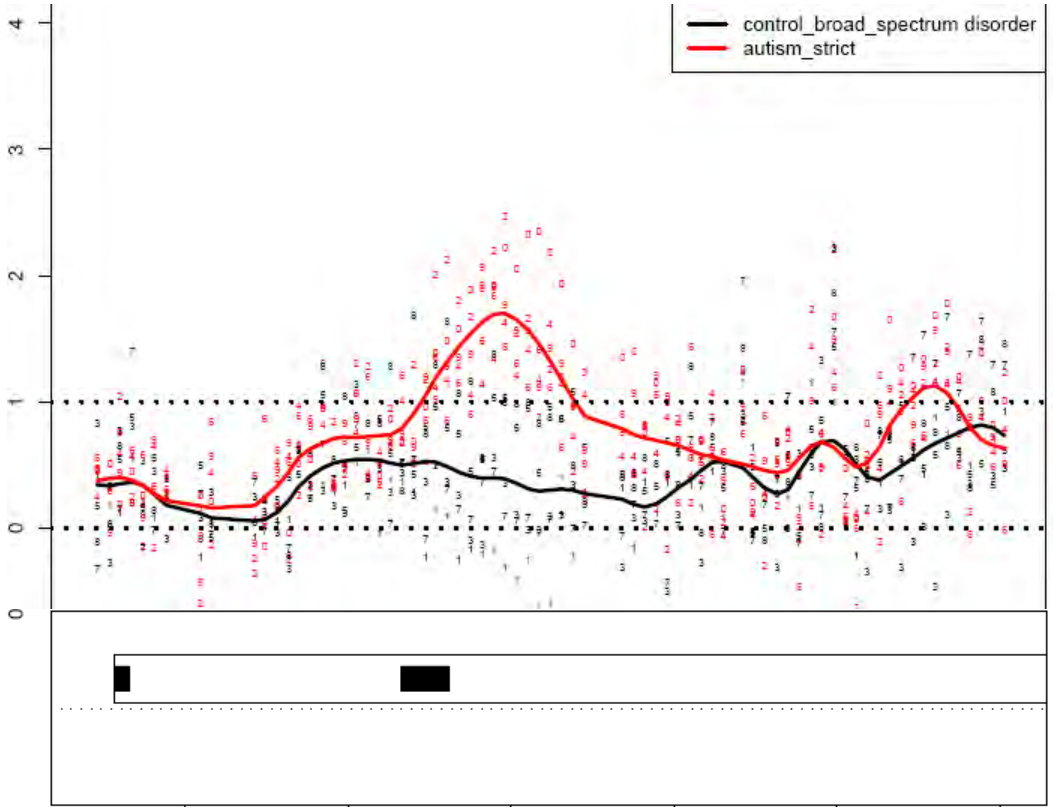
How do we approach epigenetic epidemiology?

- Major studies in our Center
  - Autism
  - Bipolar disorder
  - Major depression
  - Schizophrenia
    - Endophenotypes
    - First degree relatives
- Newborn epigenome
  - Parental genome and epigenome
  - Environmental exposure and diet
  - Neurological assessment and autism
- Sample paradigm
  - Existing cohorts with outstanding phenotype
  - GWAS
  - Twins

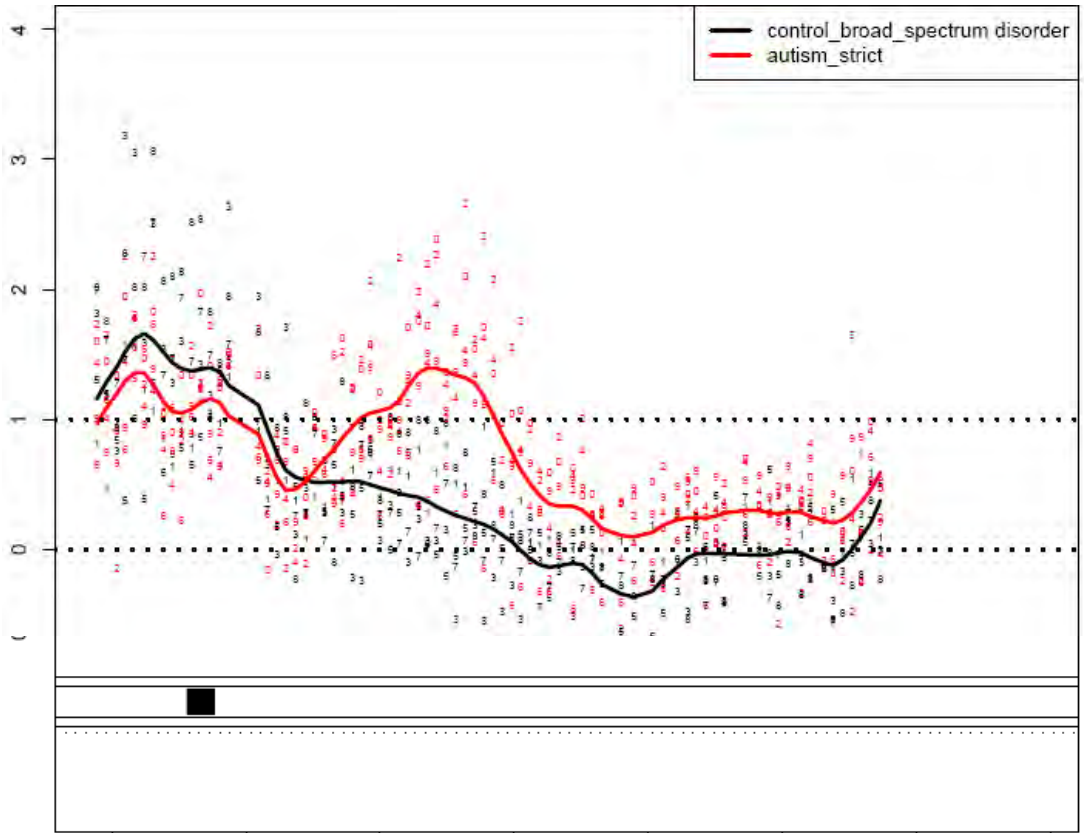
Center for Epigenetics

- School of Medicine
  - Andy Feinberg, Medicine / MBG
  - Jimmy Potash, Psychiatry
  - Sarven Sabunciyan, Pediatrics
- School of Public Health
  - Rafael Irizarry, Biostatistics
    Hongkai Ji, Ben Langmeade
  - Dani Fallin, Epidemiology
  - Lynn Goldman, Epidemiology
- Kennedy-Krieger
  - Walter Kaufmann, Neurology
- Center for Talented Youth
  - Vicky Milo
  - Lea Ybarra
- Clinical Consortia
  - Raquel Gur, Penn
  - Viswajit Nimgaonkar, Pitt
  - Rodney Go/Perry, UAB
  - David Braff, UCSD
  - Laura Almasy, SFBR
  - Doug Fugman, Rutgers
  - Craig Newschaffer, Drexel

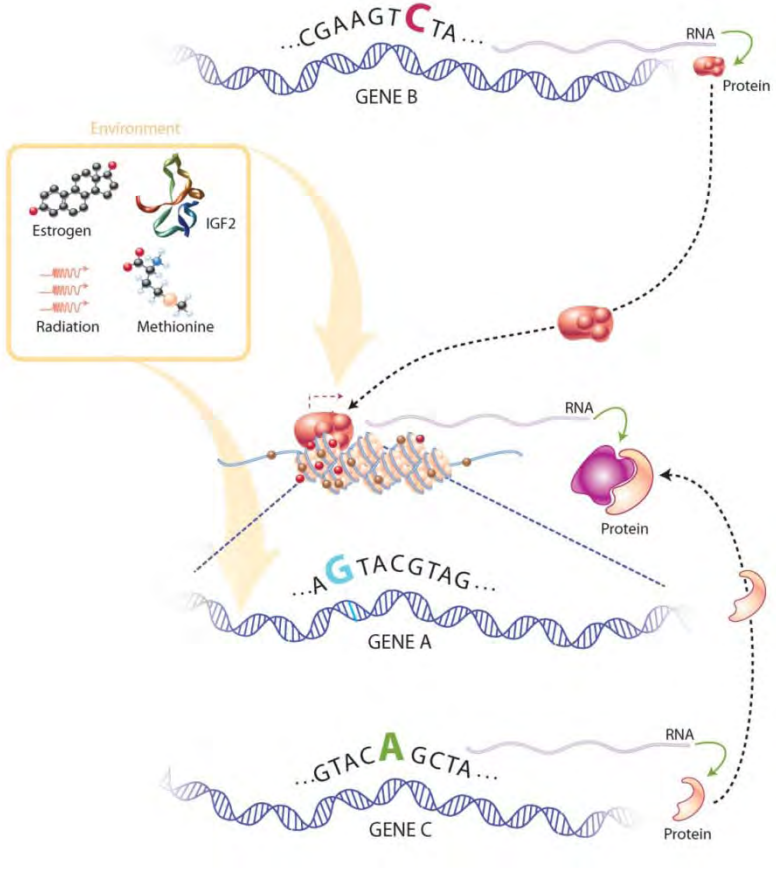
Example: male MZ twins discordant for autism



Male MZ twins discordant for autism



Epigenetic epidemiology: the common disease genetic and epigenetic (CDGE) hypothesis



- Comprehensive epigenomic analysis
  - Genome-wide methylation scan (GWM)
  - Allele-specific expression
  - Chromatin
- Population over time
- Greater subtlety of phenotype
  - Case-control
  - Quantitative
- Environmental exposures
- Epigenetic disruption causes altered phenotypic plasticity *generally*
- New field of statistical epigenetics

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  - Dani Fallin, Epidemiology
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