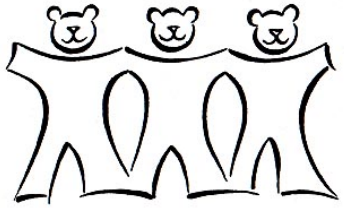
Backtracking Leukemia to Birth: Environmental Exposures and DNA Methylation Patterns in Neonatal Blood and Leukemia Cells

UCSF
University of California
San Francisco



California Childhood Leukemia Study

Joe Wiemels

University of California, San Francisco

Department of Epidemiology and Biostatistics

Our Goal: Define the causes of childhood leukemia

- Prevention
- Early Detection
- Risk stratification (treatment decisions)

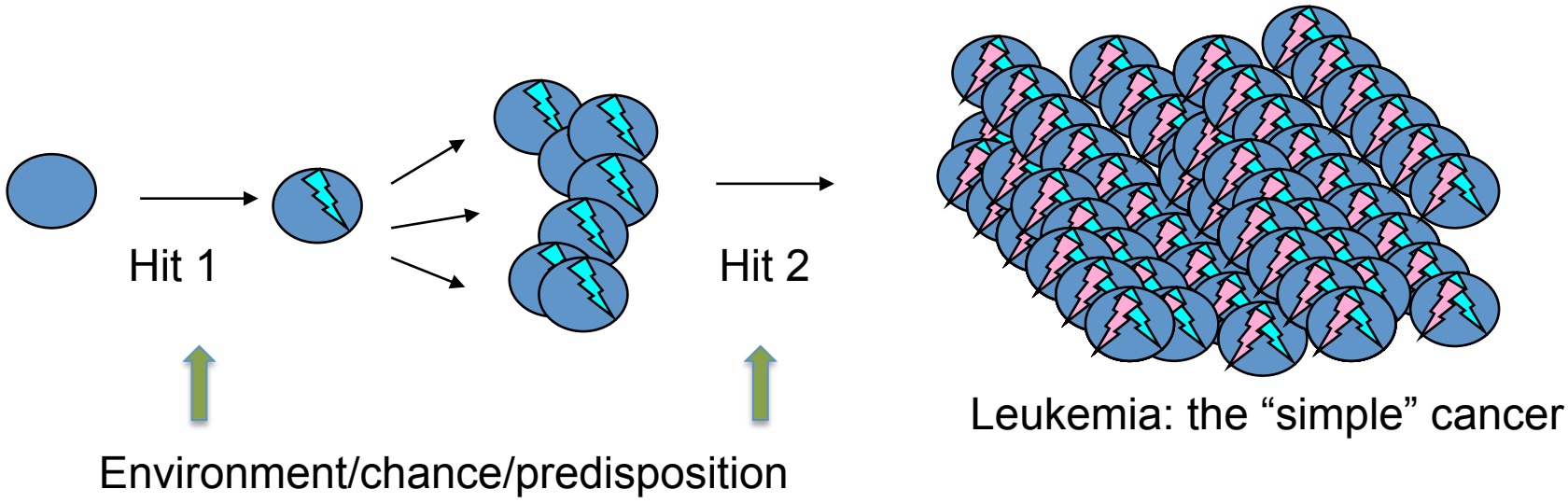
Tumor genetic/epigenetic analysis in epidemiology

- Trace the origin of the genetic and epigenetic changes that result in leukemia: timing and structure
- Use epidemiologic methodology to discover the role of environment in the formation of leukemogenic events

Etiology of Leukemia: The Model

- Childhood leukemia is a disease caused by at least two genetic/epigenetic mutations ("hits")

- These mutations will occur in clonal succession at different periods in development



Environmental Risk Factors linked to leukemia

*diagnostic X-ray exposure
diet (folate, topoisomerase II inhibitors)
infection (clusters, daycare, birth order)
birthweight
pesticides
workplace exposures, solvents
tobacco (parental)

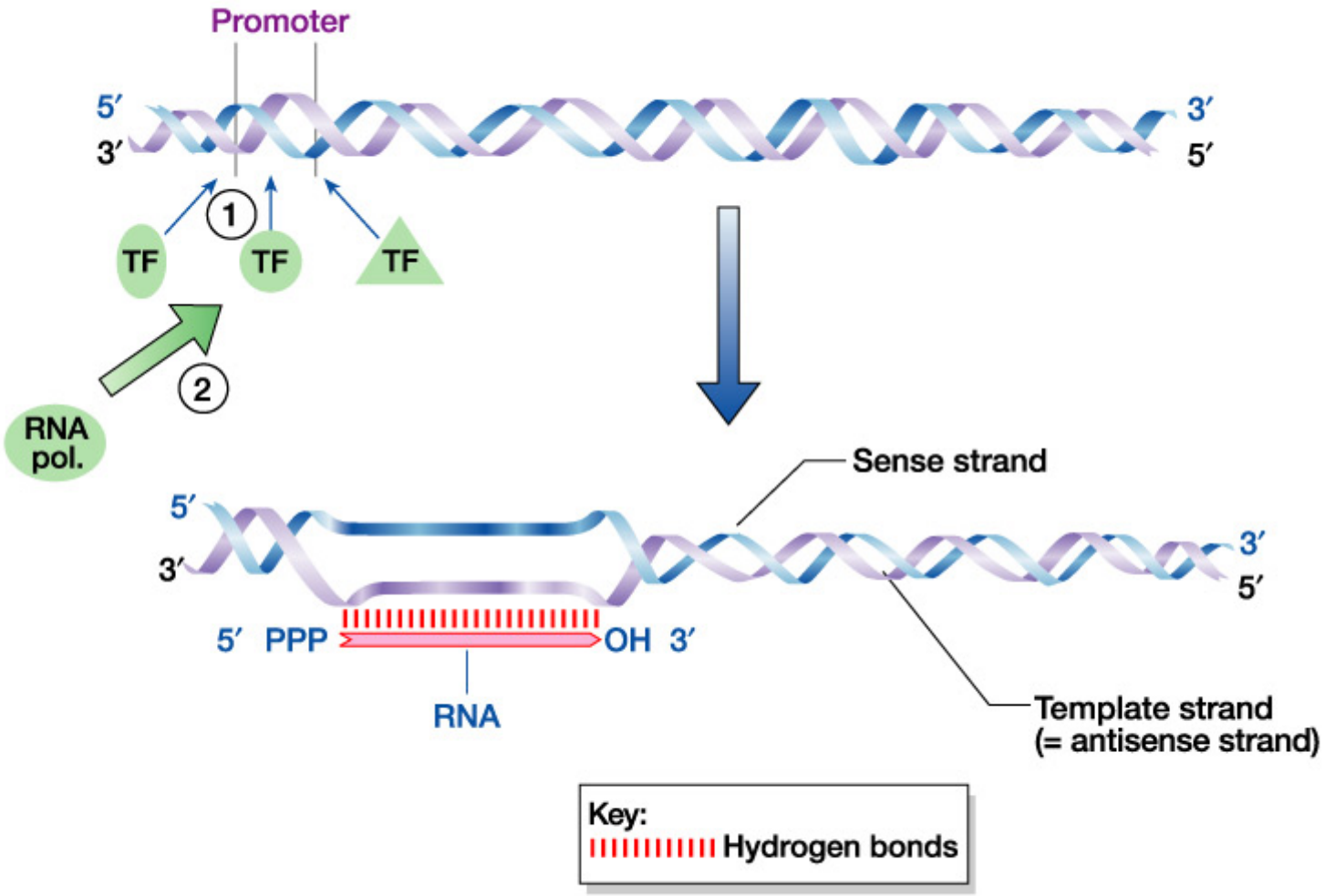
*the only generally accepted risk factor

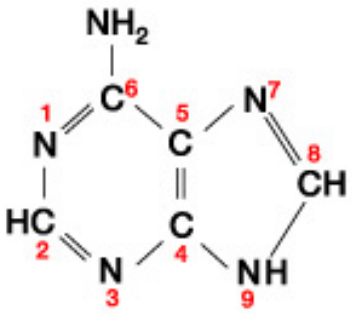
Epigenetics: DNA methylation in cancer

On a mechanistic level, epigenetics is the state of function of the genome related to gene expression which is controlled in part by the state of gene promoters

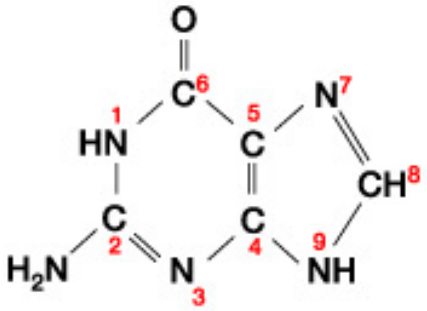
Gene expression levels are dependent on promoter function.

Epigenetics is the control of promotor accessibility to transcription factors.

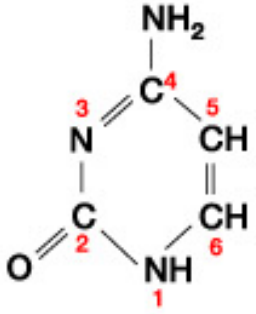




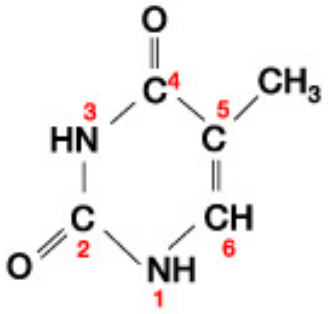
Adenine (A)



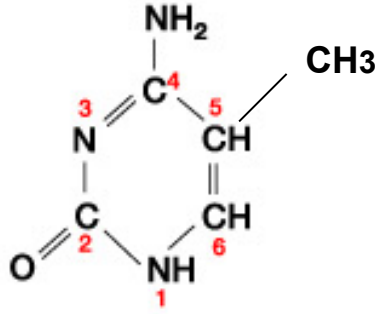
Guanine (G)



Cytosine (C)



Thymine (T)



5-methyl- Cytosine (C)

The “fifth base”

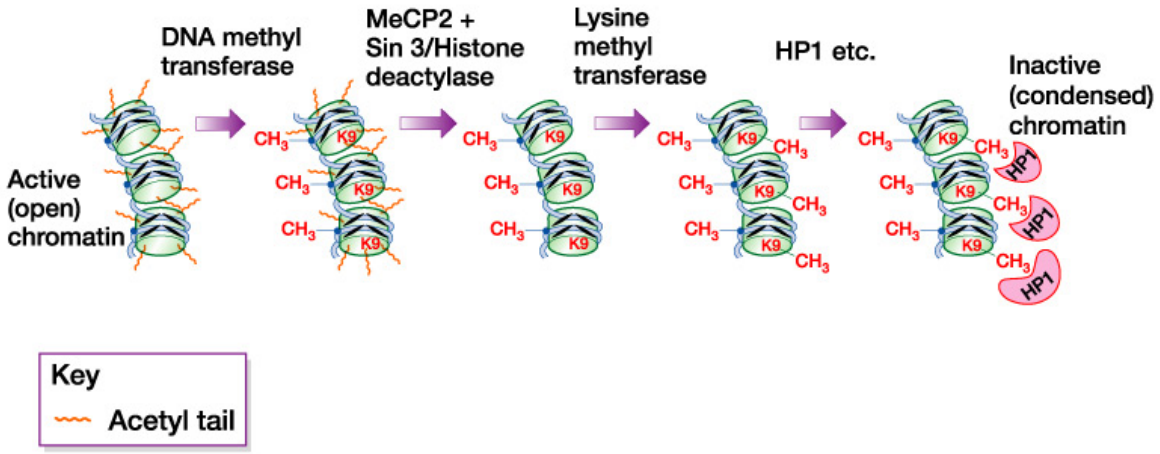
5-methyl C facts

Only occur at CpG sites 5′-GT**CG**TAACAT**CG**ATGGCA-3′

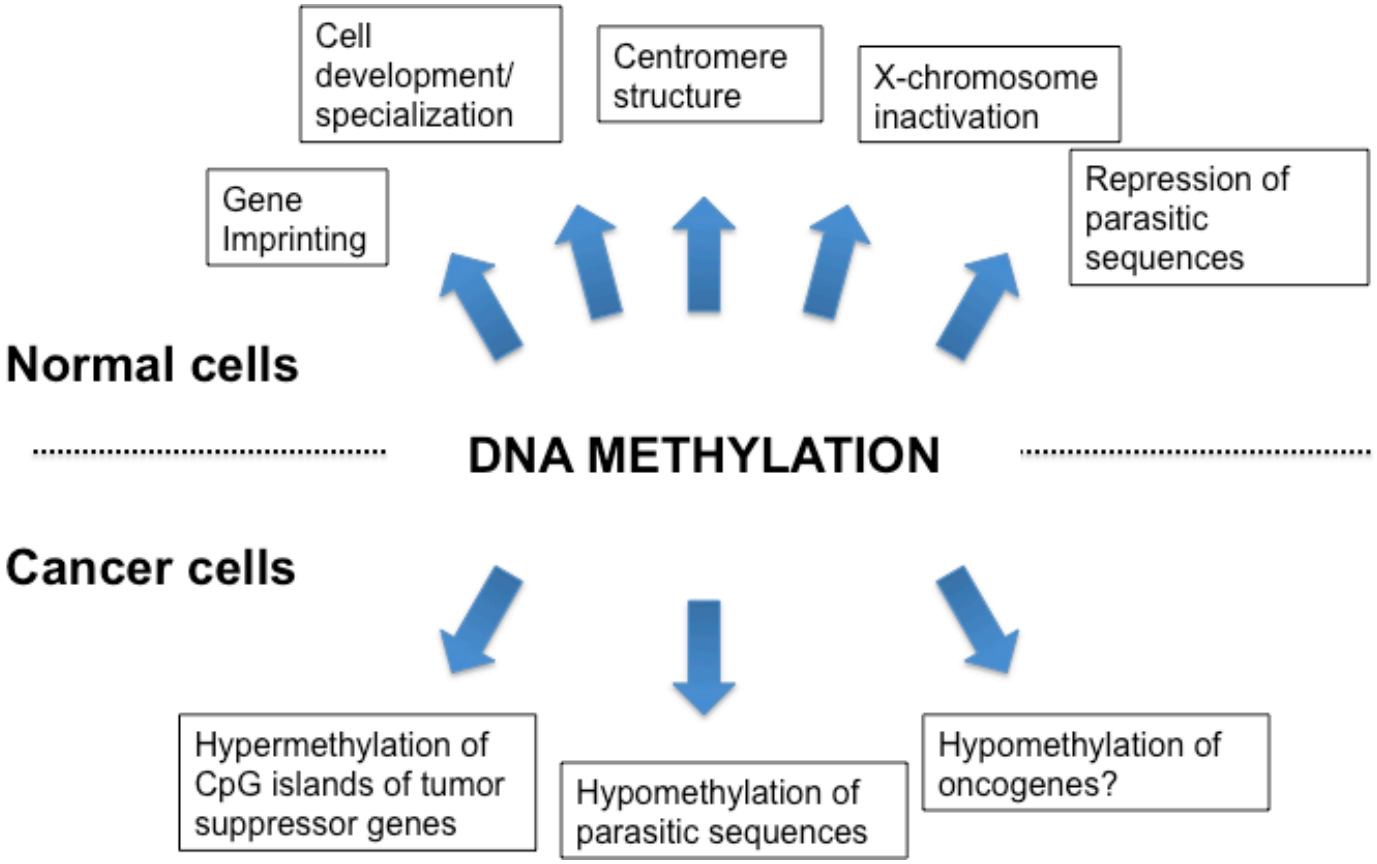
Most CpGs are methylated in the genome, and associated with interspersed repeat sequences (“parasitic” DNA) and heterochromatin

CpGs that occur in high density are associated with promoter regions and are typically unmethylated. 45,000 such “CpG islands” exist.

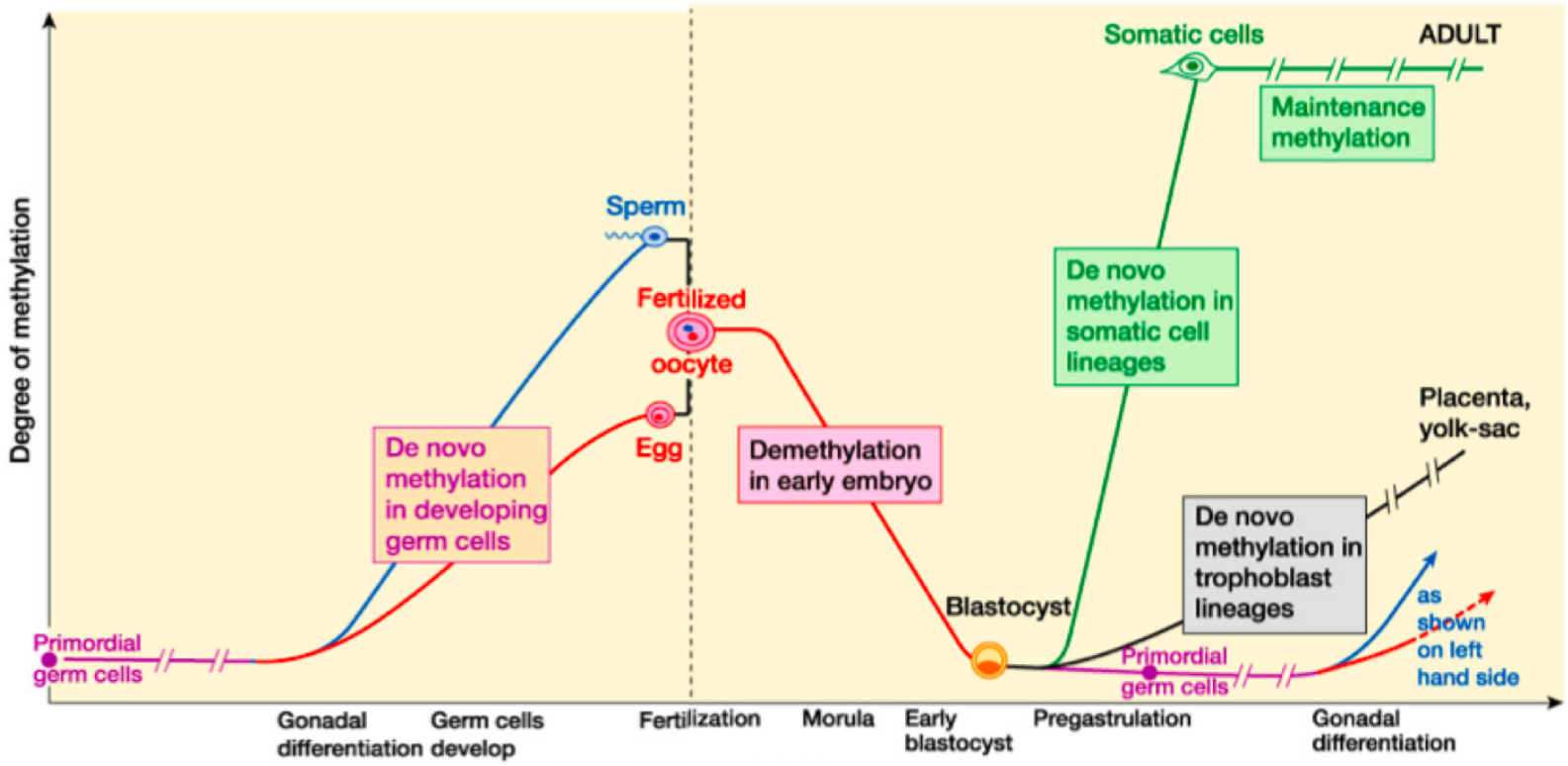
Unmethylated CpG associated with open chromatin state.



DNA methylation in normal development and cancer



DNA methylation in development



DNA methylation is metastable during development

DNA methylation: environmental exposures and disease

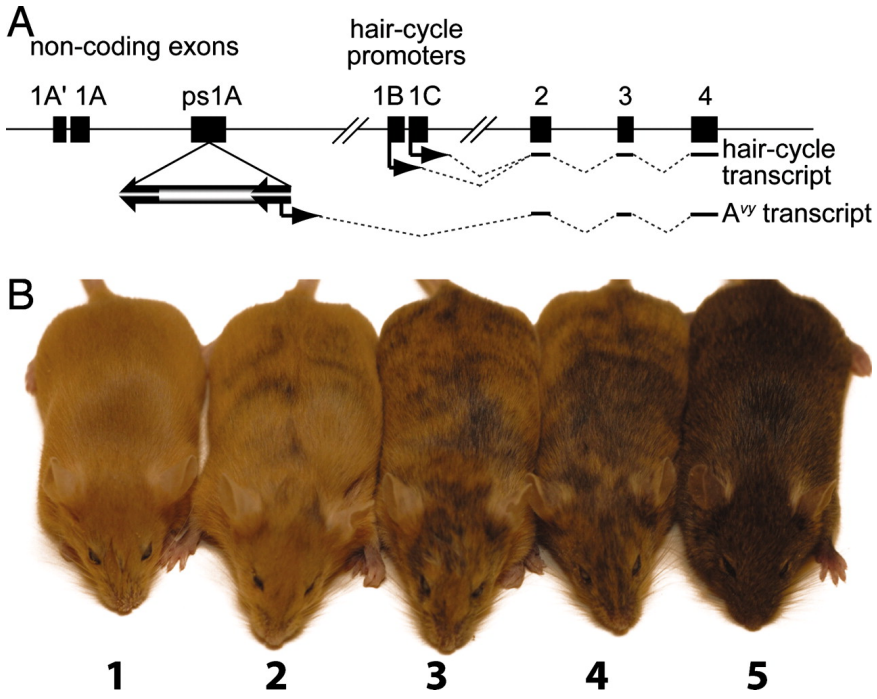
We can measure and classify everyone

High dimension methodology

“sensor” of environmental insults and also highly relevant to the disease

Exposure → **Biomarker** → Disease

Gene expression permanently influenced by pregnancy folate



Cropley, PNAS **103 46: 17308-17312, 2006**

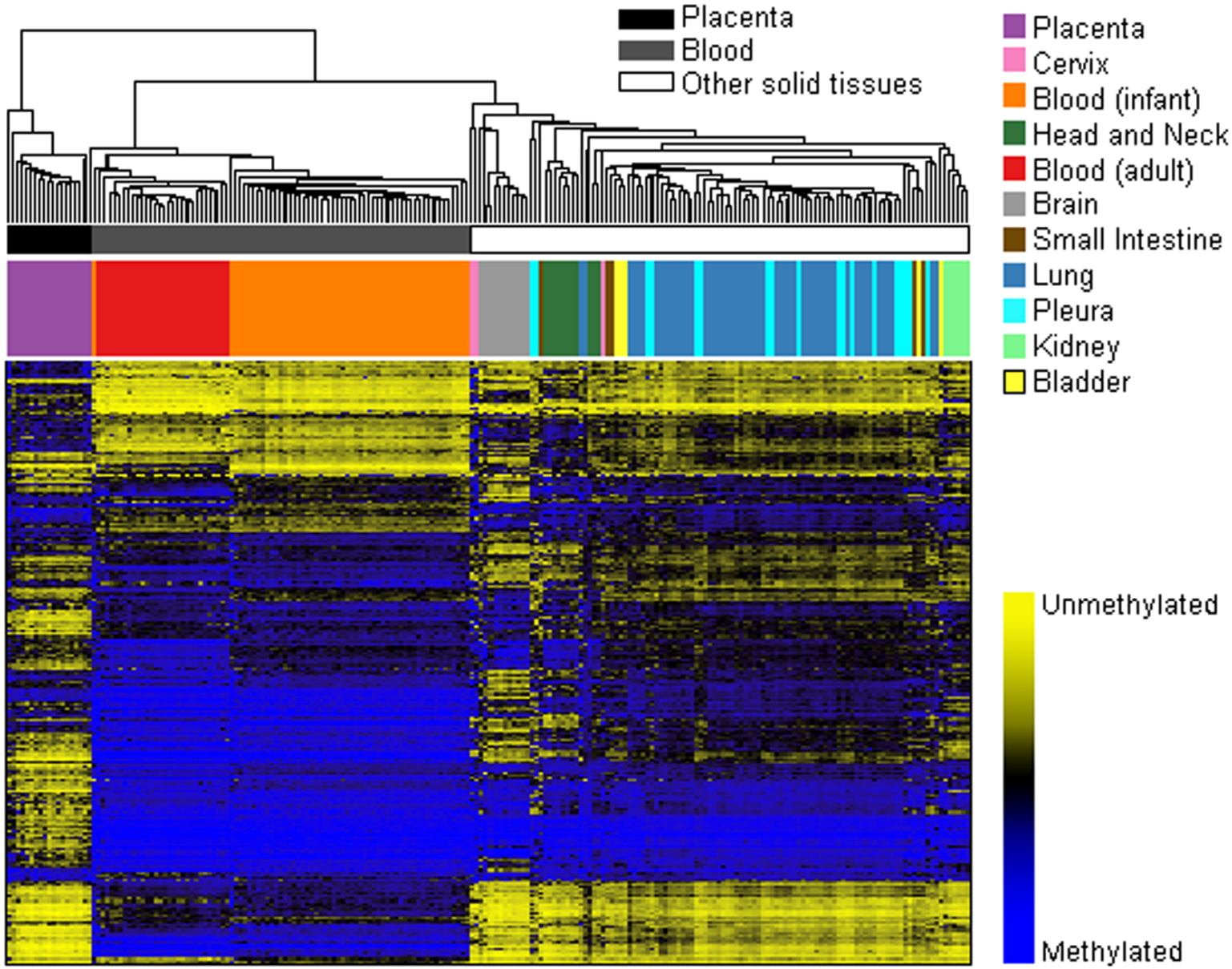
- **These mice are clones with identical DNA sequence**

Illumina GoldenGate Arrays

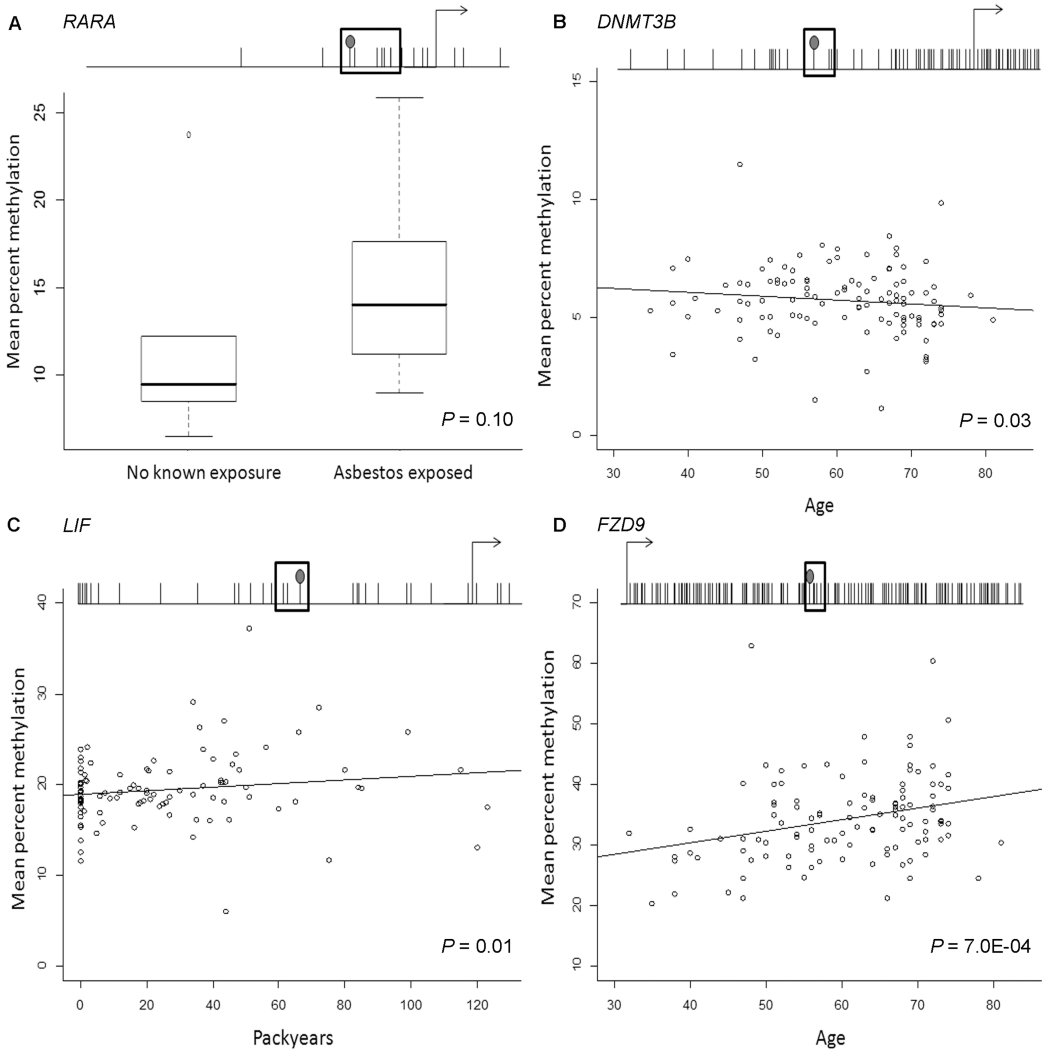
- **1505 loci associated with over 800 cancer-related genes**
- **Essentially it is a genotyping assay for CpG loci, but statistically a continuous measurement from 0 to 1.**
- **Distinguishing methylated from unmethylated sequence is achieved through bisulfite modification of genomic DNA**

Cytosine converts to Uracil
Methyl-Cytosine remains cytosine

Different normal tissues have different DNA methylation patterns



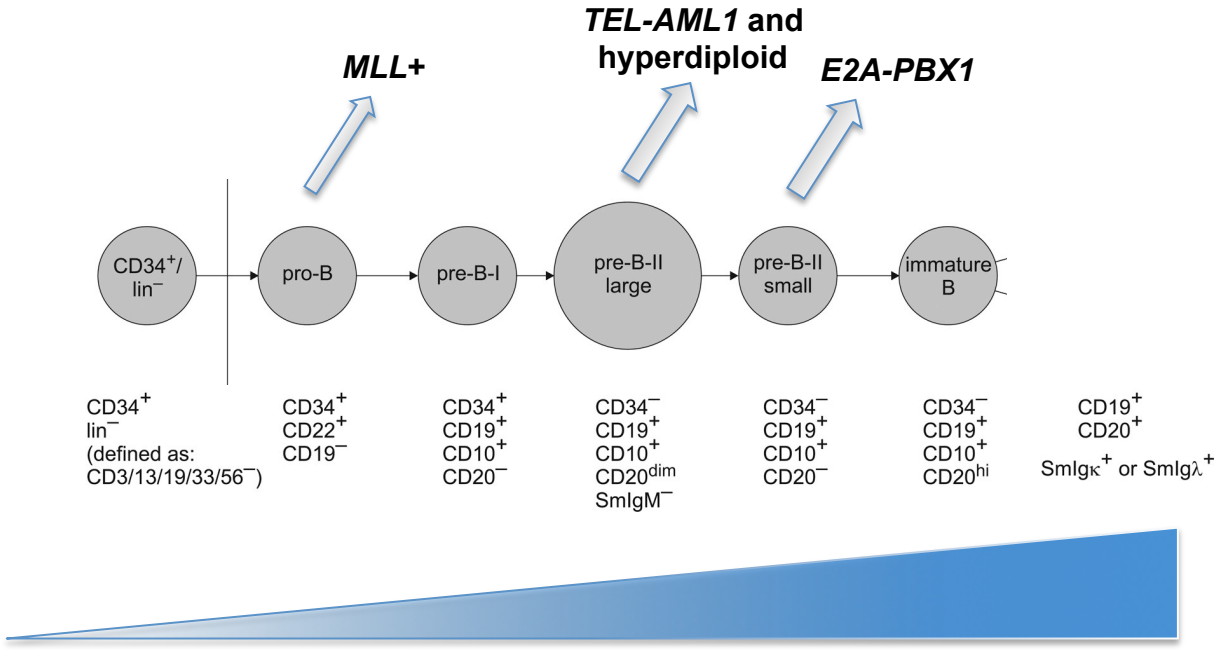
Relationships between epidemiologic variables and DNA methylation in normal blood



Bisulfite pyrosequencing: quantitative measurement of DNA methylation at a single CpG site

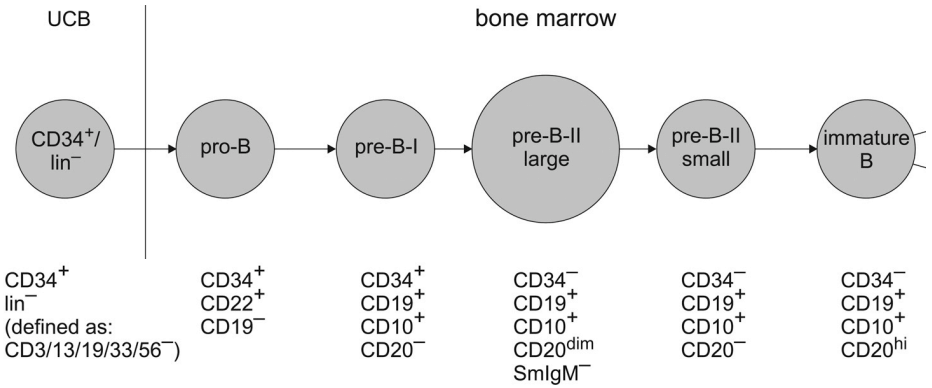
Christensen, PLOS Genetics 2009

DNA methylation during pre-B cell development

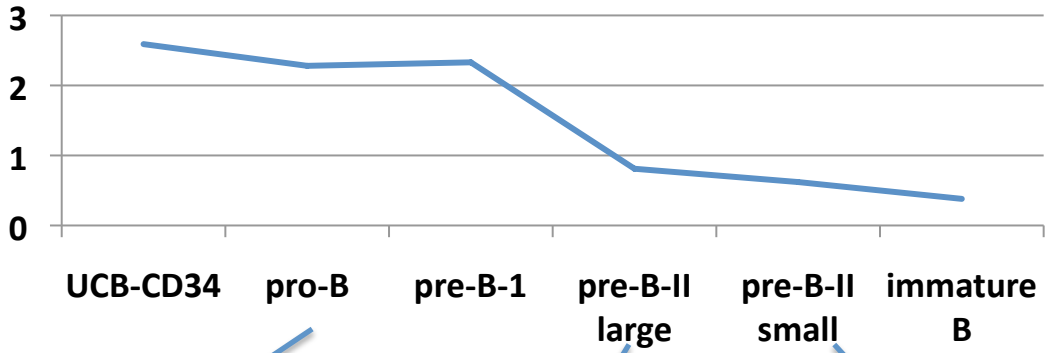


DNA methylation in blood cell development

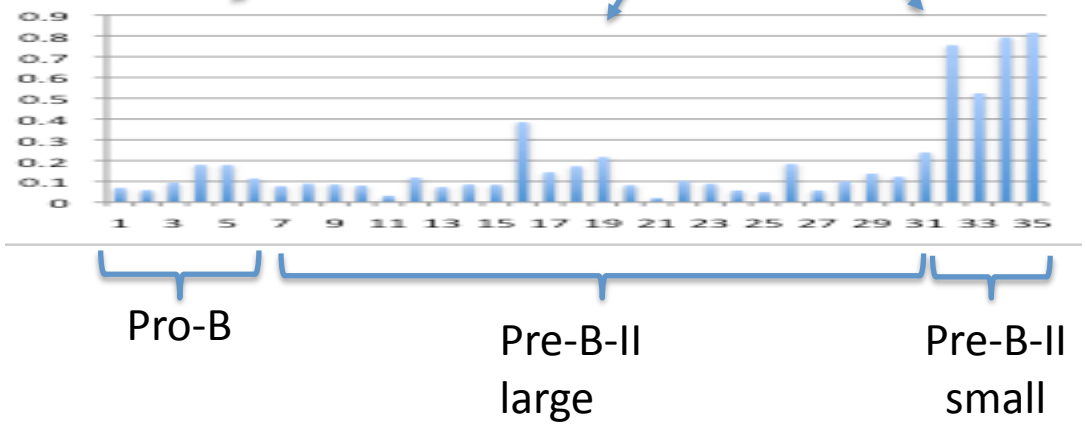
A



B



C



CD34 expression in leukemia

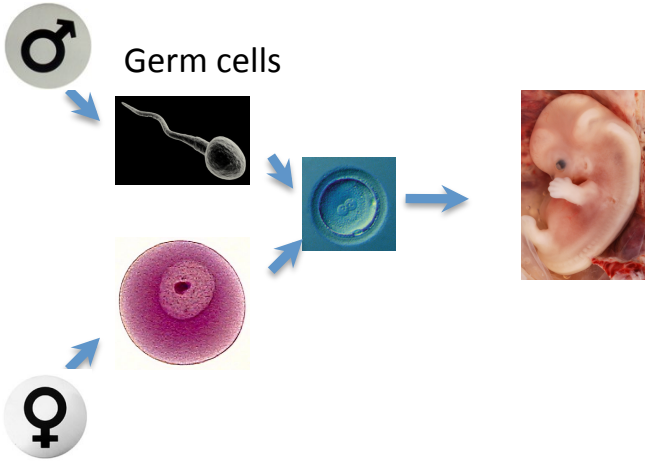
CD34 methylation in leukemia

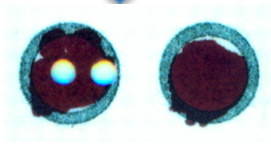
DNA methylation in Guthrie cards (case-control)

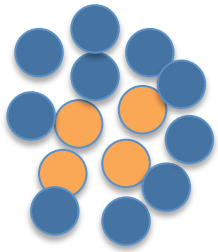
DNA methylation profiles of leukemia (case only)

Folate nutrition, parental smoking, pesticides

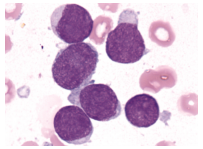
Infection, daycare, vaccination

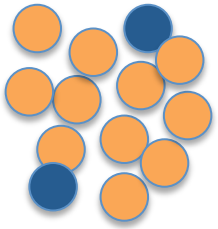






Guthrie, birth blood



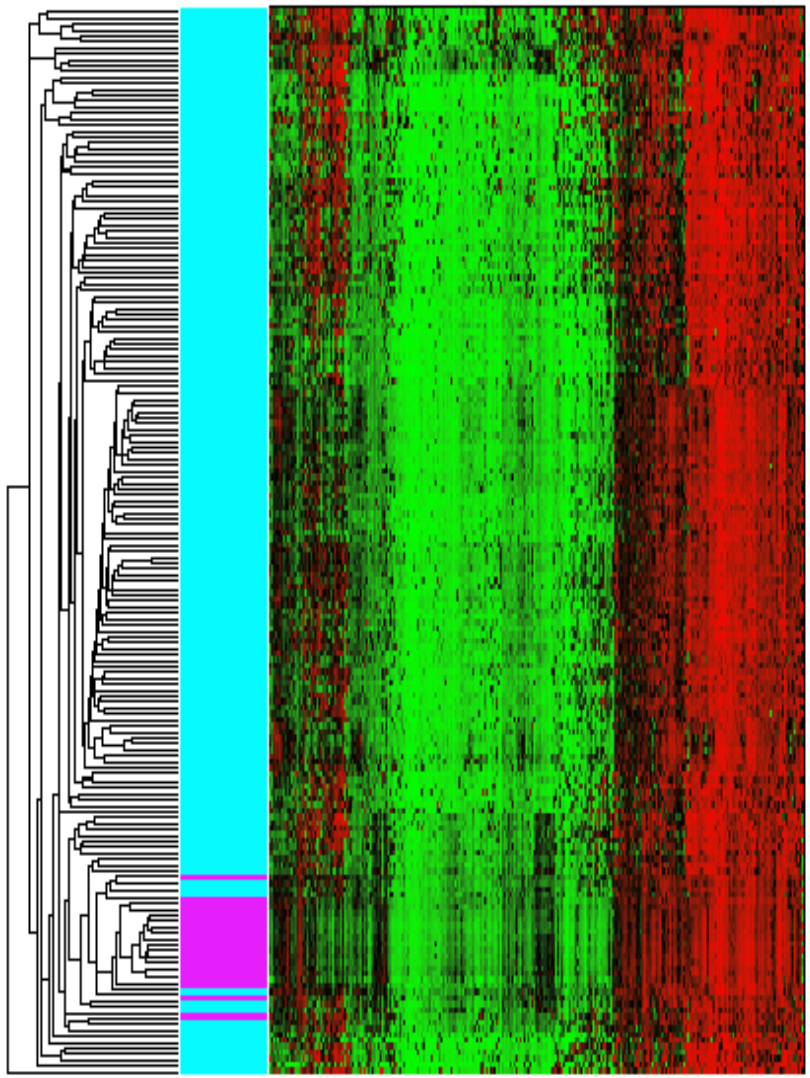


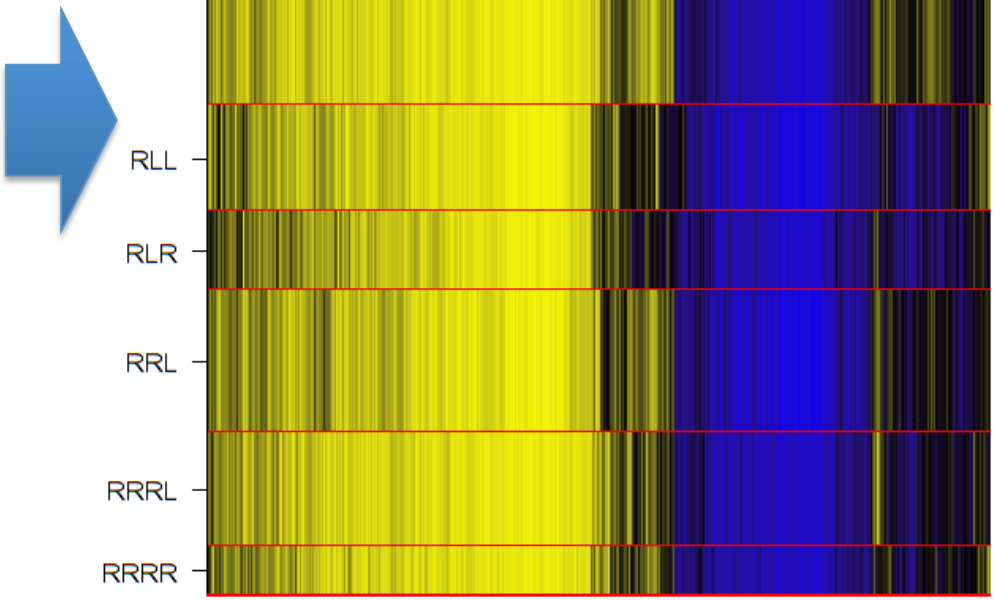
Bone marrow, clonal leukemia

Recursive Partition Methylation Modeling (RPMM)

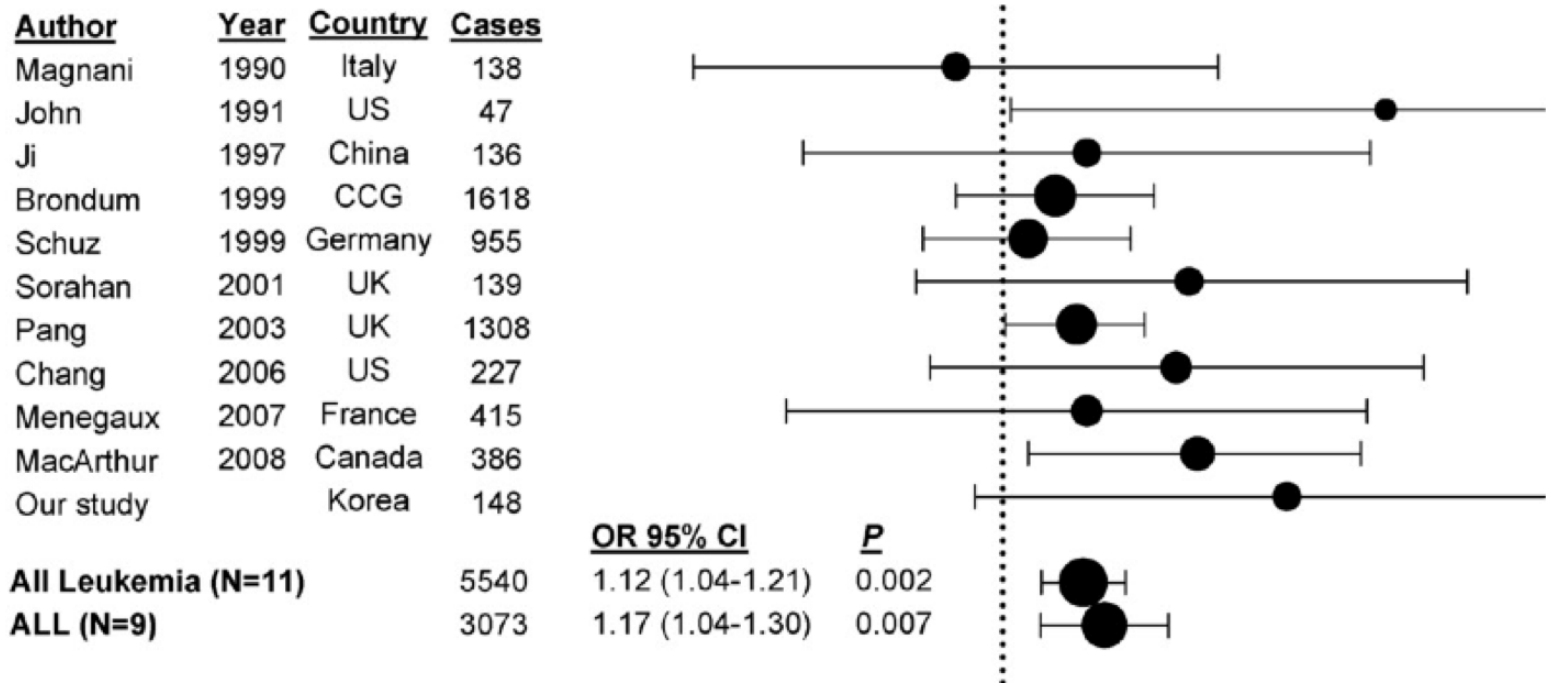
- Select the number of clusters in a data set
- beta mixture model
- unsupervised variant of Hierarchical Mixtures of Experts, a fuzzy version of CART
- Model-based version of HOPACH algorithm
- Samples are “split” when the data better fits by Bayesian information criteria
- Andres Houseman, Brock Christensen et al., *BMC Bioinformatics*

RPMM analysis



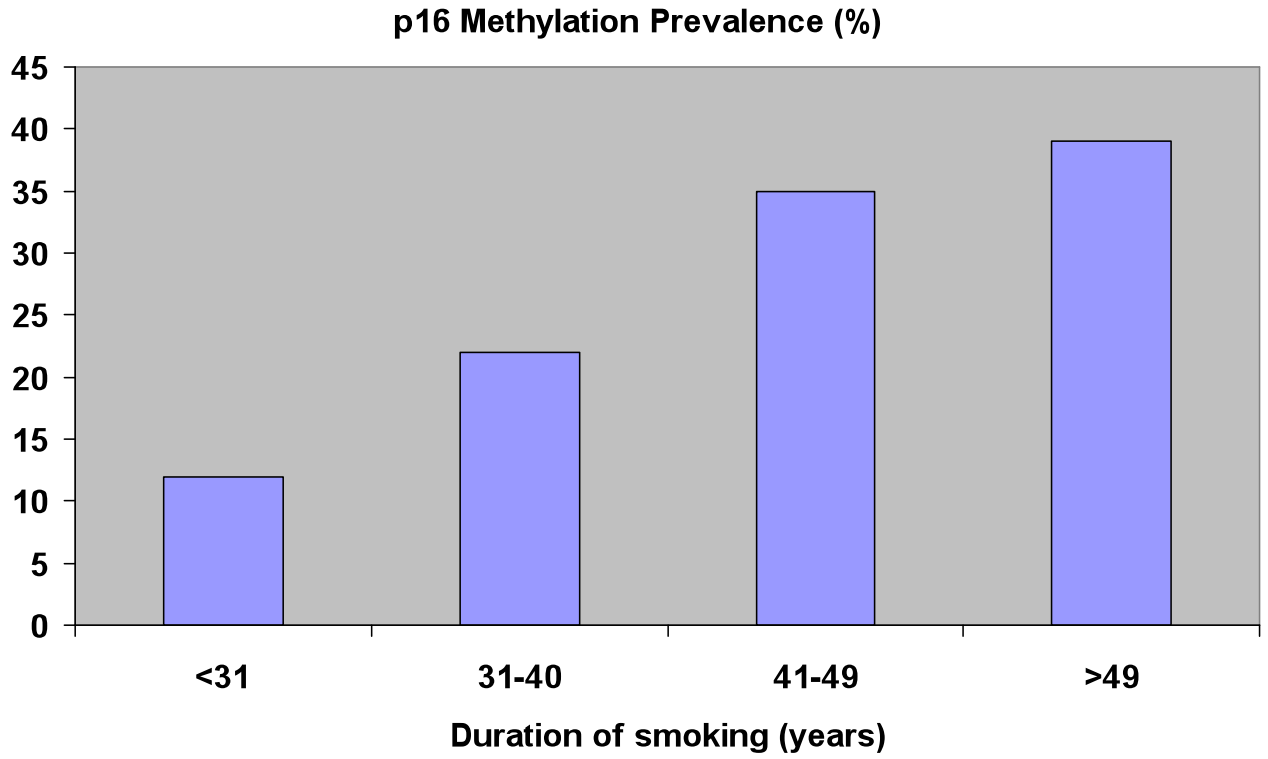


Paternal smoking (and not maternal) is a risk factor for childhood leukemia



Epidemiology of parental smoking and childhood leukemia: Kang et al., 2008

Prior evidence: tobacco and p16 gene methylation in lung



Kim, D.-H. et al. Cancer Res 2001

Infection and childhood leukemia

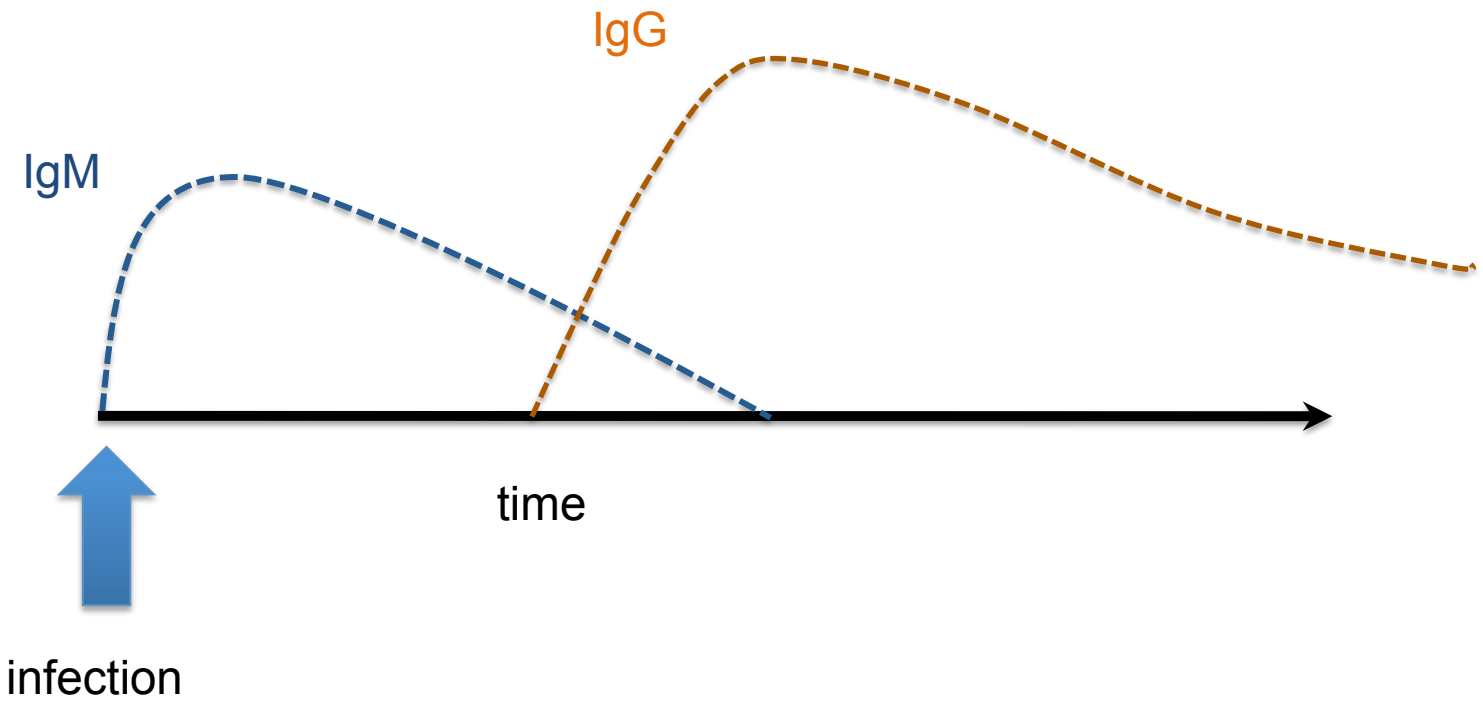
- Early, common infections are *protective*
- Late infections (proximal to diagnosis) are *risk factors*
- No specific infection has been associated with leukemia
- Is there an "imprint" of viral involvement left behind?

→the proverbial "hit and run"?

Parvovirus B19

- “fifth” disease of childhood, common and ubiquitous.
- Causes fever and rash or occasionally no symptoms
- Erythrocyte precursors involved

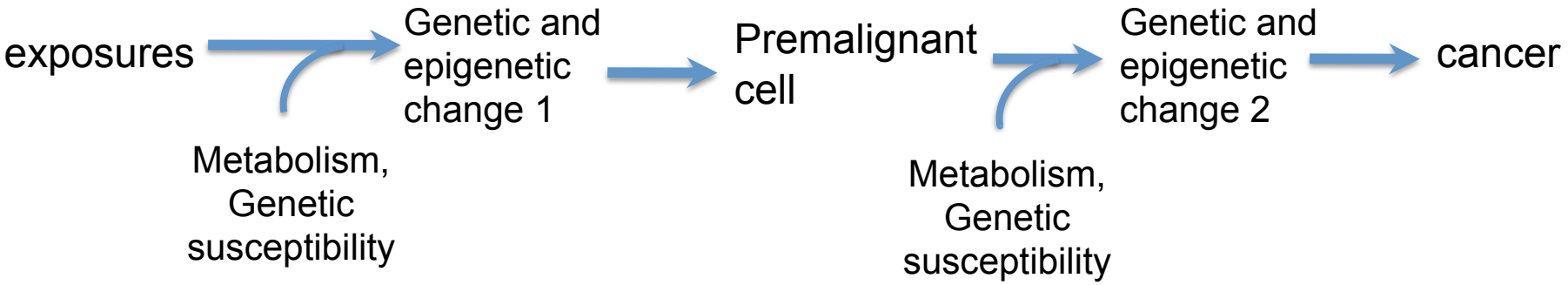
IgM and IgG in an immune reaction



Summary

- Epigenetic aberrations can occur in early development and contribute to cancer
  - May lead to prediction and risk stratification
- Environment appears to have a profound effect on DNA methylation patterns
- For research, an intensive environmental assessment is *crucial*

Summary



- Genetic and epigenetic markers contribute to disease, and can be caused directly or indirectly by exposures

Can be used for:

- Tracing the origins of disease
- Sub-classification of cancers in etiologically-relevant subgroups

Acknowledgements

UCB:

Patricia Buffler

Catherine Metayer

Anand Chokkalingam

Luoping Zhang

Monique Does

Karen Bartley

Cliona McHale

Jeffrey Chang

Martyn Smith

INCA, Brazil

Gisele Vasconcelos

Maria Pombo de-Oliveira

University of Minnesota

Heather Nelson

UCSF:

Sheng Zhong

John Wiencke

Yuanyuan Xiao

Shichun Zheng

Margaret Wrensch

Murdoch, Melbourne

Nicholas Wong

ICR, UK

Mel Greaves

Brown University:

Brock Christensen

Andy Houseman

Karl Kelsey

Carmen Marsit

Dartmouth

Margaret Karagas

Physicians and Participants in the California Childhood Leukemia Study

Funders: NCI, NIEHS, EPA, TRDRP, LLS, CwL