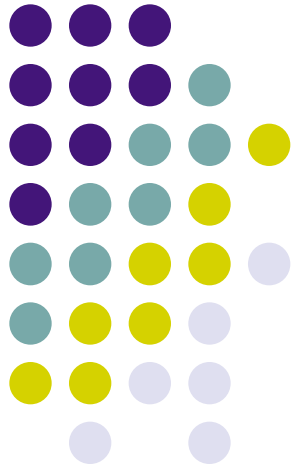
Epigenetics: Epicenter of Modern Medicine?

By Guillaume Pare MD
Assistant professor
McMaster University



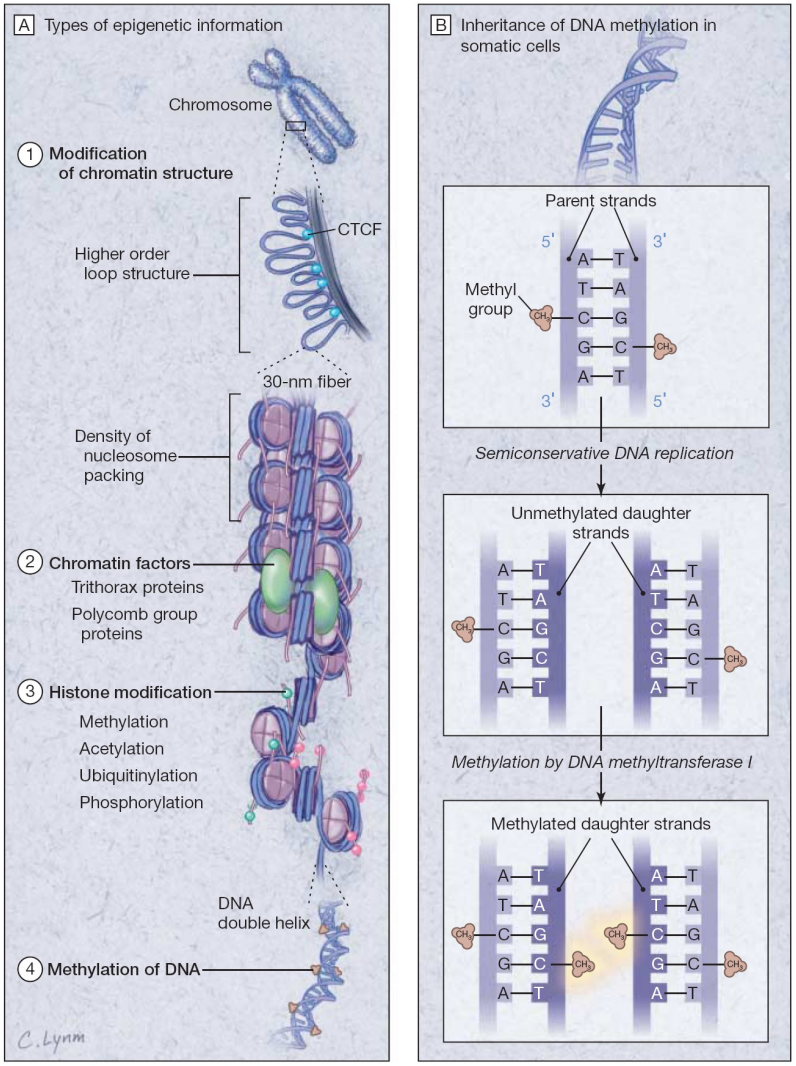
Definitions

- “Transmission of information not encoded in DNA sequences from cell to daughter cell or from generation to generation”

- “Mitotically or meiotically heritable changes in gene expression that do not involve a change in DNA sequence”

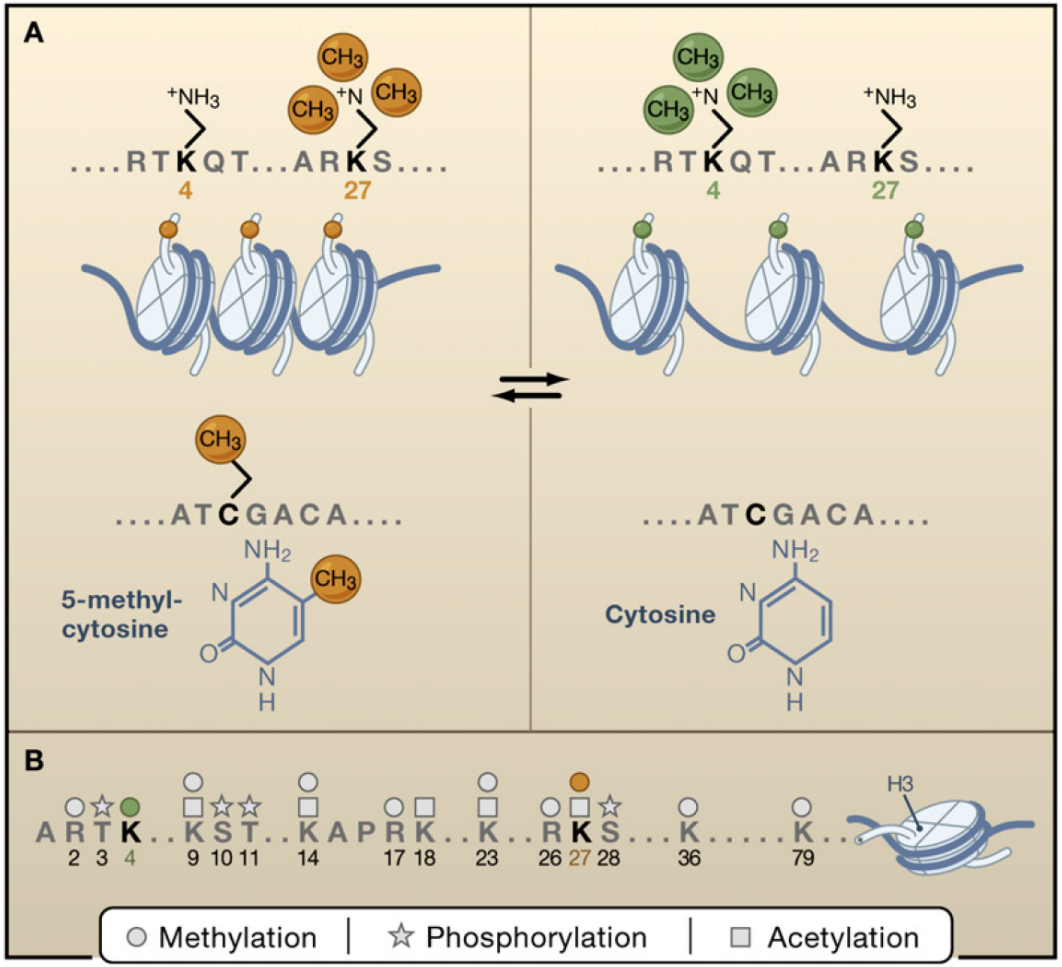
- “Modifications of the DNA or associated proteins, other than DNA sequence variation, that carry information content during cell division”

Mechanisms



Feinberg et al. JAMA 2008; 299: 1345-1350

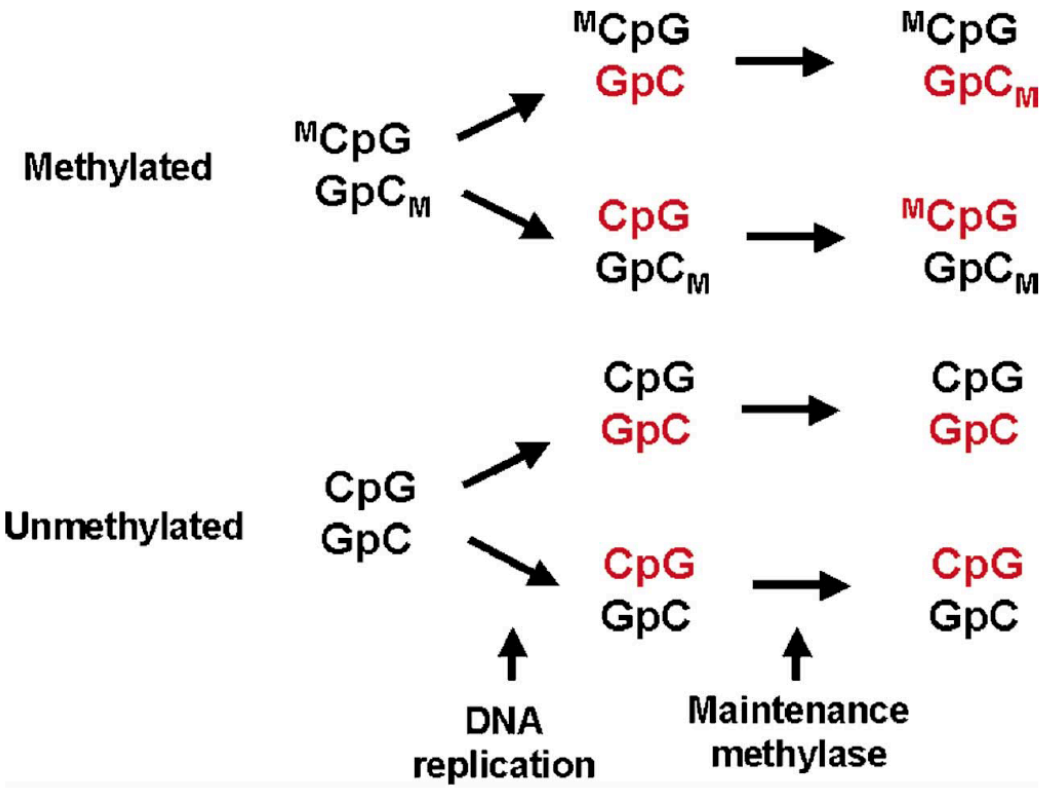
Mechanisms



Bernstein et al., Cell 2007; 128, 669–681

Mechanisms

- Transfer of methylation from cell to daughter cell:



Ann Rev Gen Hum Gen 2004; 5:479-510

Mechanisms

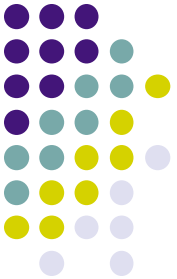
- Methylation is widespread in the human genome, except for…

- CpG Islands
  - Defined by GC content >55%, observed to expected CpG frequency >0.6, and >500 bp
  - Hypomethylated
  - Enriched in genes promoters (56% of genes)
  - ~12% are methylated, which leads to repression of gene expression

Functions

- Why epigenetics?
  - Gene expression regulation:
    - DNA methylation represses gene expression
    - Explains the phenotypic differentiation of cells with the exact same sequence of DNA
    - Complex regulation at the histone level…
  - Inhibits transposon replication (?)
  - Innate immunity (?)

How to measure epigenetic changes?

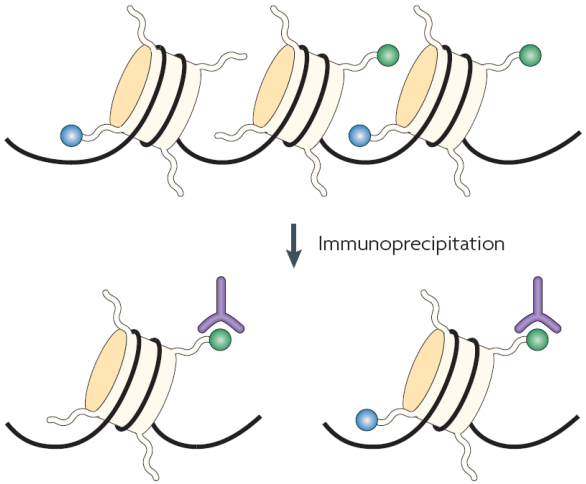


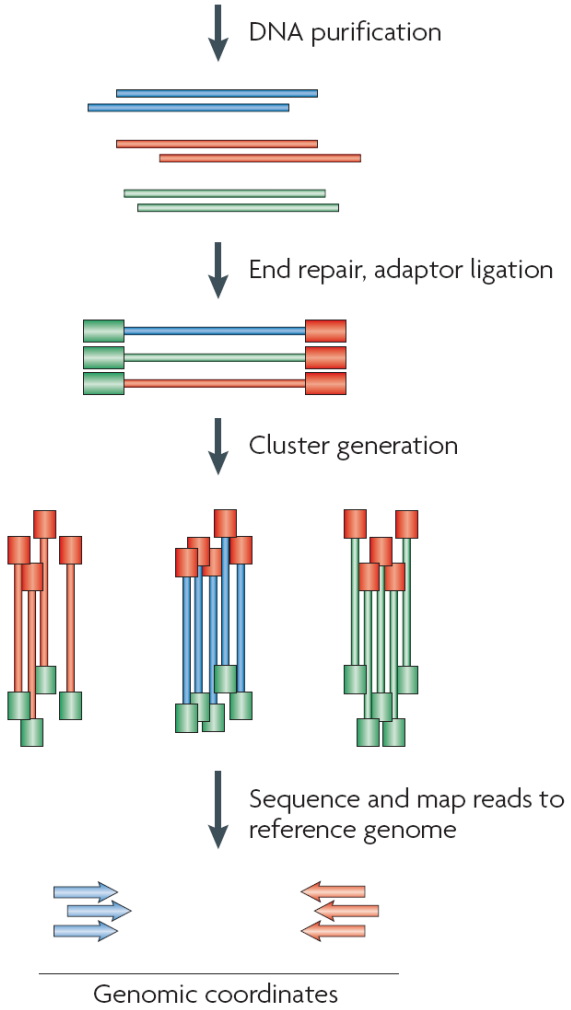
- Discrimination methods:
  - Methylation sensitive restriction enzymes
  - Affinity (antibodies or binding proteins)
  - Bisulfite sequencing

- Detection methods:
  - Gel electrophoresis
  - Sanger sequencing
  - DNA tiling arrays
  - SNP genotyping
  - Next-generation sequencing sequencing

How to measure epigenetic changes?

Chromatin Immunoprecipitation (ChIP)





Advantages:

-Can detect all types of epigenetic changes
-Amenable to whole-genome screening
-Can use a variety of detection methods

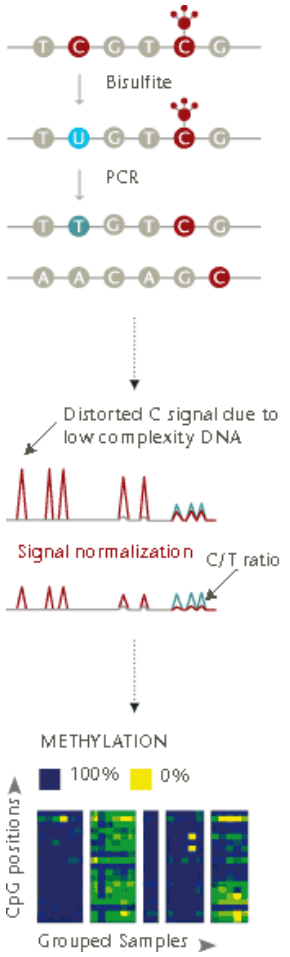
Disadvantages:

-Technically challenging
-Dependant on the antibody
-Low resolution

How to measure epigenetic changes?

- Bisulfite sequencing:

Advantages:
-Single base-pair resolution
-Amenable to whole-genome screening
-Can use a variety of detection methods
-Easy to use
Disadvantages:
-Only for methylation



Description

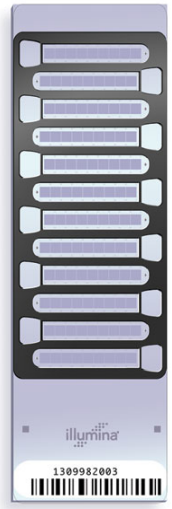
1. Genomic DNA is converted using Epigenomics' proprietary bisulfite pre-analytics workflow

2. PCR products are directly sequenced. Sequencing trace file are normalized. Relative methylation levels are calculated by determining the ratios between C (red) and T (green) signals at individual CpG positions.

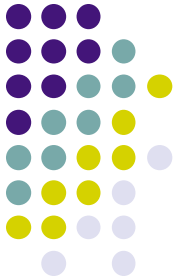
3. Calculated methylation ratios at individual CpG positions are translated into a color-coded data matrix

How to measure epigenetic changes?

- Cutting-edge methods:
  - Array based methylation analysis (Bisulfite)
    - Technology based on SNP genotyping
    - Coverage of >450K CpG loci
    - Low cost (!)

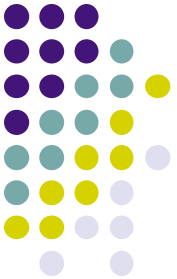


  - Bisulfite with next-generation sequencing
  - ChIP-Seq (next generation sequencing)
  - Mass spectrometry for histone modifications



Epigenetics and Disease

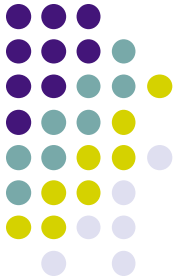
Epigenetics and Disease



- First example in 1983 with the widespread loss of DNA methylation in colorectal cancer
  - Cancer as a genetic disease
  - De-differentiation

- Single gene disorders of the epigenetic machinery
  - Rett syndrome (MeCP2)
  - Immunodeficiency, centromeric instability and facial anomalies (DNMT3B)

Genomic Imprinting



- “The epigenetic marking of a locus on the basis of parental origin, which results in monoallelic gene expression”
- In other words:
  - Imprinting is a special case of epigenetics
  - Occurs when either the maternal or paternal allele is exclusively expressed
  - Established in the parental gametes
  - ~1% of all genes are imprinted!

Genomic Imprinting

- ‘Parental conflict hypothesis’
  - Paternally expressed genes tend to increase fetal growth
  - Maternally expressed genes tend to decrease fetal growth
  - Therefore controls the amount of resources that are extracted from the mother by her offspring
  - Thought to have occurred because of polygamy, viviparity and substancial maternal investments in the offspring without a similar level of investment by the father

Genomic Imprinting

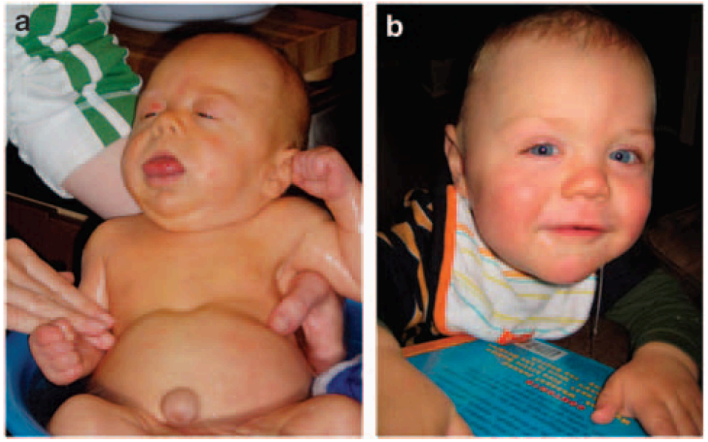
TABLE 3 Selected disorders of imprinted genes

Disorder	Mechanisms in approximate order of frequency	Chromosome region	Specific gene(s)	OMIM
Prader-Willi syndrome	Deletion, UPD, imprint defect	15q11-q13	snoRNAs?, other?	176270
Angelman syndrome	Deletion, UPD, imprint defect, point mutation	15q11-q13	*UBE3A*	105830
Beckwith-Wiedemann syndrome	Imprint defect, UPD, duplication, translocation, point mutation	11p15.5	*IGF2*, *CDKN1C*	130650
Pseudohypoparathyroidism	Point mutation, imprint defect, UPD	20q13.2	*GNAS*	103580
Russell-Silver syndrome	UPD, duplication, translocation, inversion	7p11.2	Various candidates	180860

Ann Rev Gen Hum Gen 2004; 5:479-510

Genomic Imprinting

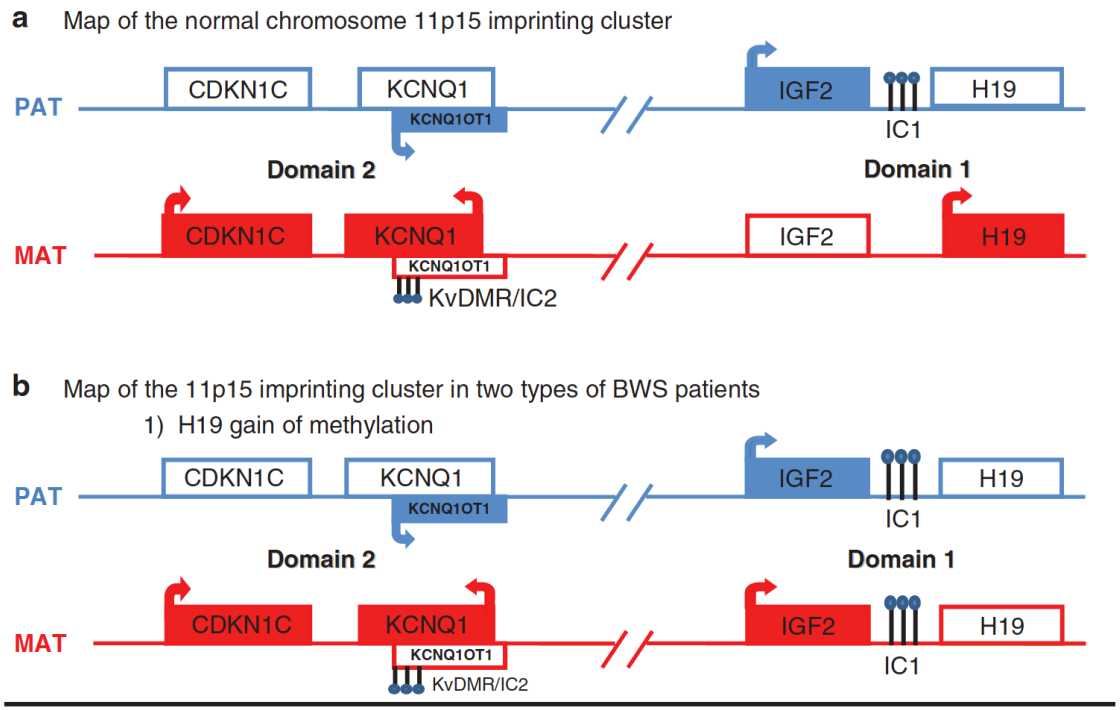
- Beckwith-Wiedemann Syndrome:
  - Clinical characteristics:
    - Pre-natal overgrowth
    - Midline abdominal wall defects
    - Ear creases or pit
    - Neonatal hypoglycemia
    - Wilms and other embryonal tumors



  - Caused by LOI of the IGF2 (11p15.5) gene in 15% of cases

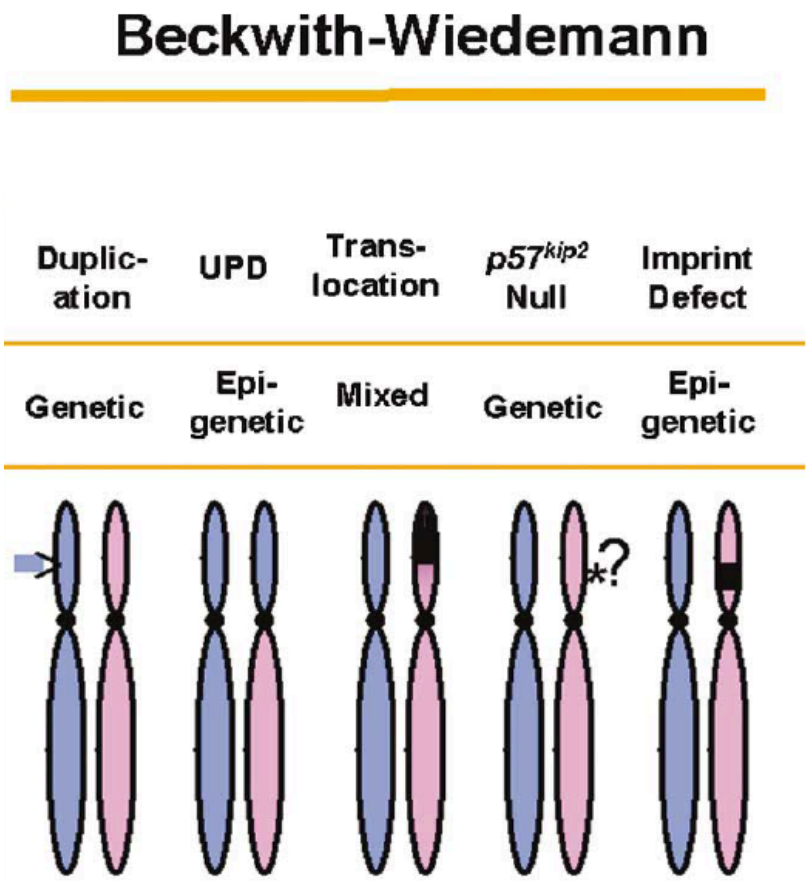
Ann Rev Gen Hum Gen 2004; 5:479-510

Genomic Imprinting



Weksberg et al. Eur J Hum Gen 2010; 18: 8-14

Genomic Imprinting



Ann Rev Gen Hum Gen 2004; 5:479-510

Genomic Imprinting

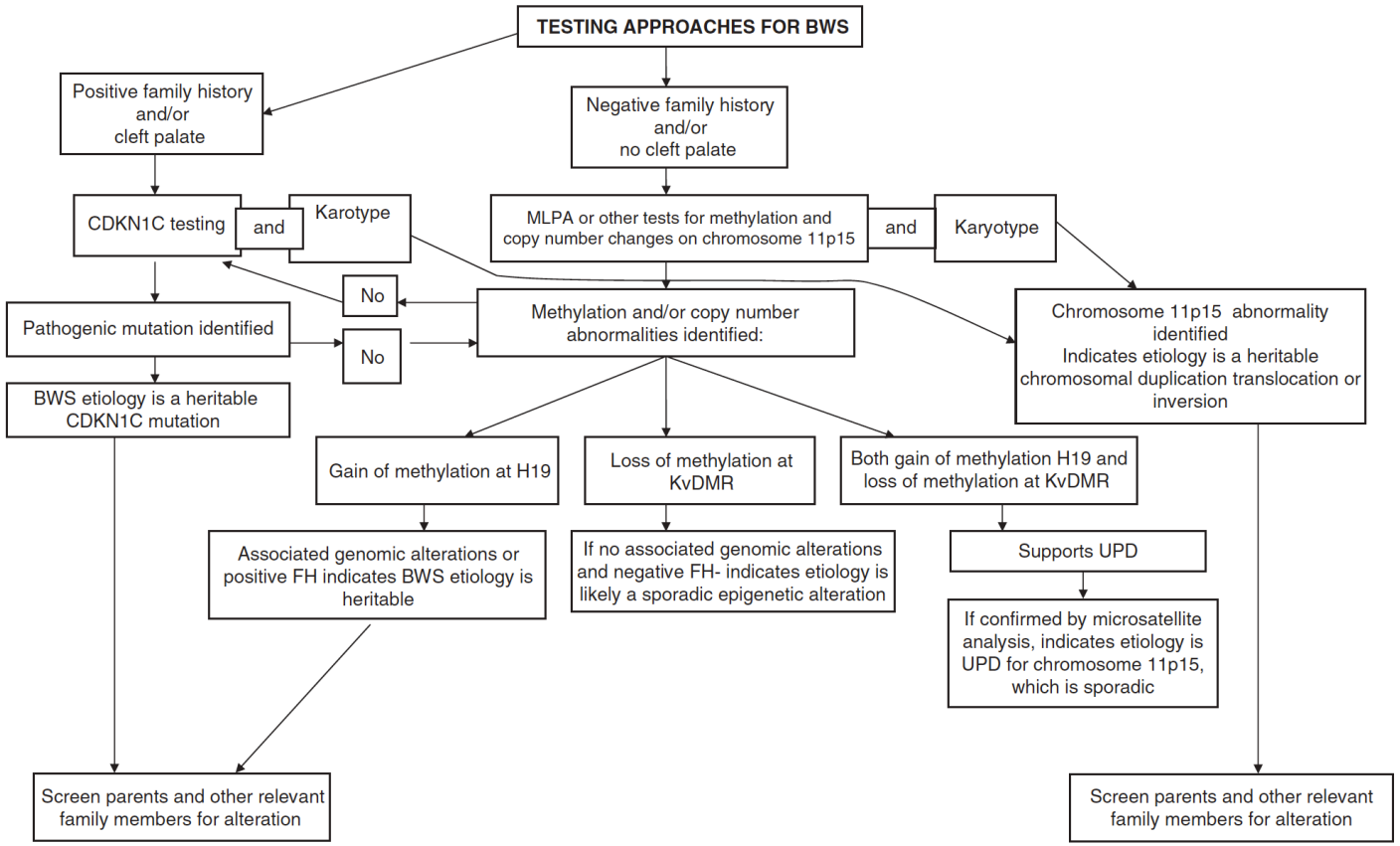
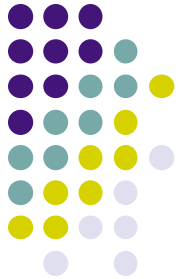


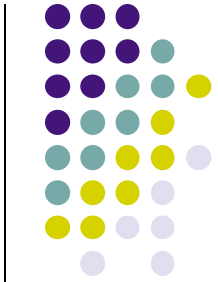
Figure 3 A clinical approach to testing for BWS.

Weksberg et al. Eur J Hum Gen 2010; 18: 8-14

Genomic Imprinting

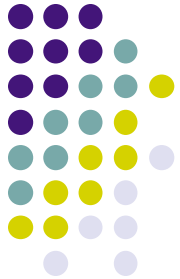


- Interestingly, lymphocytes in approximately 10% of the human population have LOI at the IGF2 locus
  - Strongly correlated with IGF2 LOI in normal colonic mucosa
  - Itself a strong risk factor (OR ~ 5) for colorectal cancer
  - Has been proposed as a biomarker…



Complex Traits and Epigenetics

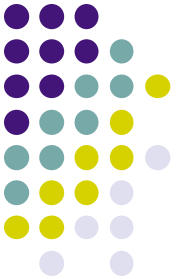
Common Disease Genetic and Epigenetic Hypothesis



"In addition to genetic variation, epigenetics provides an added layer of variation that might mediate the relationship between genotype and internal and external environmental factors"

Feinberg et al. JAMA 2008; 299: 1345-1350

Common Disease Genetic and Epigenetic Hypothesis



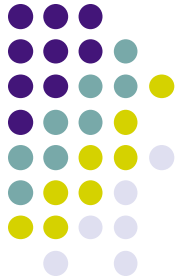
- In other words, environmental factors modifying epigenetic marks on the DNA result in loss of phenotypic plasticity characteristic of aging

- …But are environmental factors really modifying epigenetic marks on the DNA??

Complex Traits and Epigenetics

- Monozygotic twins
  - Same DNA sequence
  - Similar but not truly identical phenotype
  - Epigenetic marks thought to explain differences
  - Greater variance of DNA methylation and histone modification in older monozygotic twins than in younger twins
  - Twins who had spent their life apart had the greatest difference

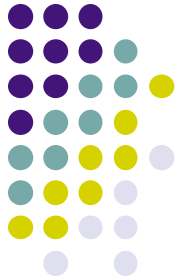
Complex Traits and Epigenetics



- MZ Vs DZ twins
  - Classical model to study genetic effects
  - Intra-pair correlation MZ >> DZ
  - …But depends on whether the twins are dichorionic (first 4 days) or monochorionic
  - Apparently, genetic variation plays a negligible role in explaining these differences

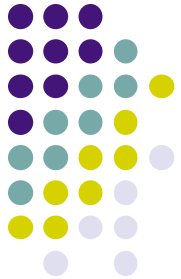
Kaminsky et al. Nat Gen 2009; 41: 240-5

Complex Traits and Epigenetics



- Dietary methionine and DNA methylation
  - Methionine is an essential amino acid that is converted to a biologically active donor state through a pathway also involving folate, B6 and B12
  - Necessary for DNA methylation
  - In mice, reduction of dietary methionine affects coat color by altering DNA methylation of the *agouti* gene

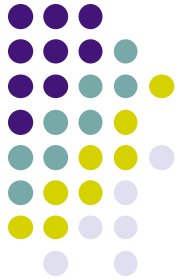
Complex Traits and Epigenetics



- Agouti encodes a paracrine molecule that promotes follicular melanocytes to produce yellow phaeomelanin rather than the black pigment

- Normally expressed only transiently during hair growth in hair follicules, resulting in brown (agouti) coat color

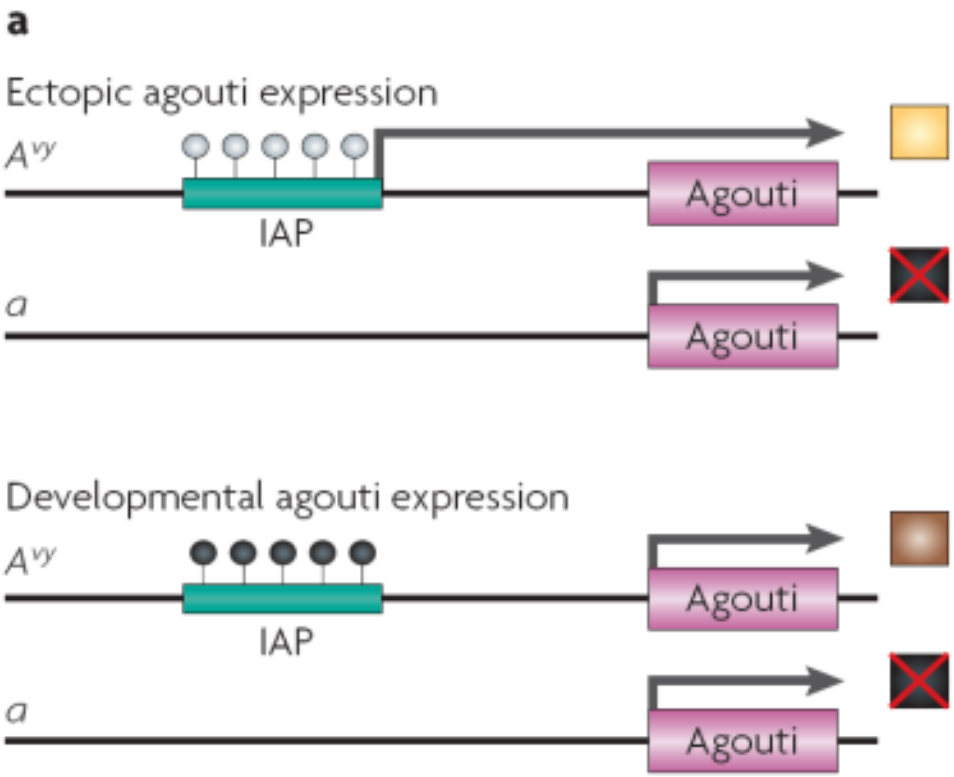
Complex Traits and Epigenetics

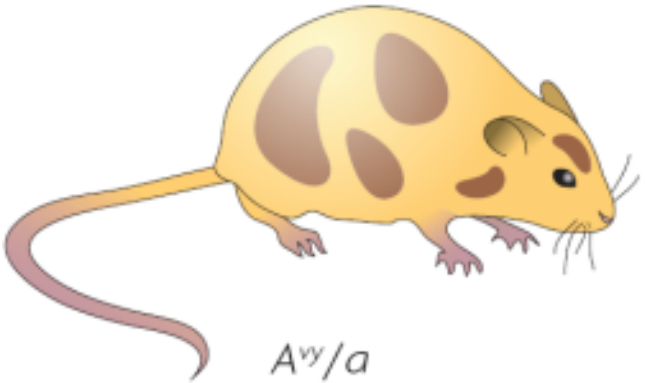


- A cryptic promoter induces constitutive expression of agouti, leading to yellow fur, diabetes, obesity and tumorigenesis

- The degree of methylation of the cryptic promotor varies dramatically among individuals, causing wide distribution in coat color, from yellow (un-Me) to brown (Me)

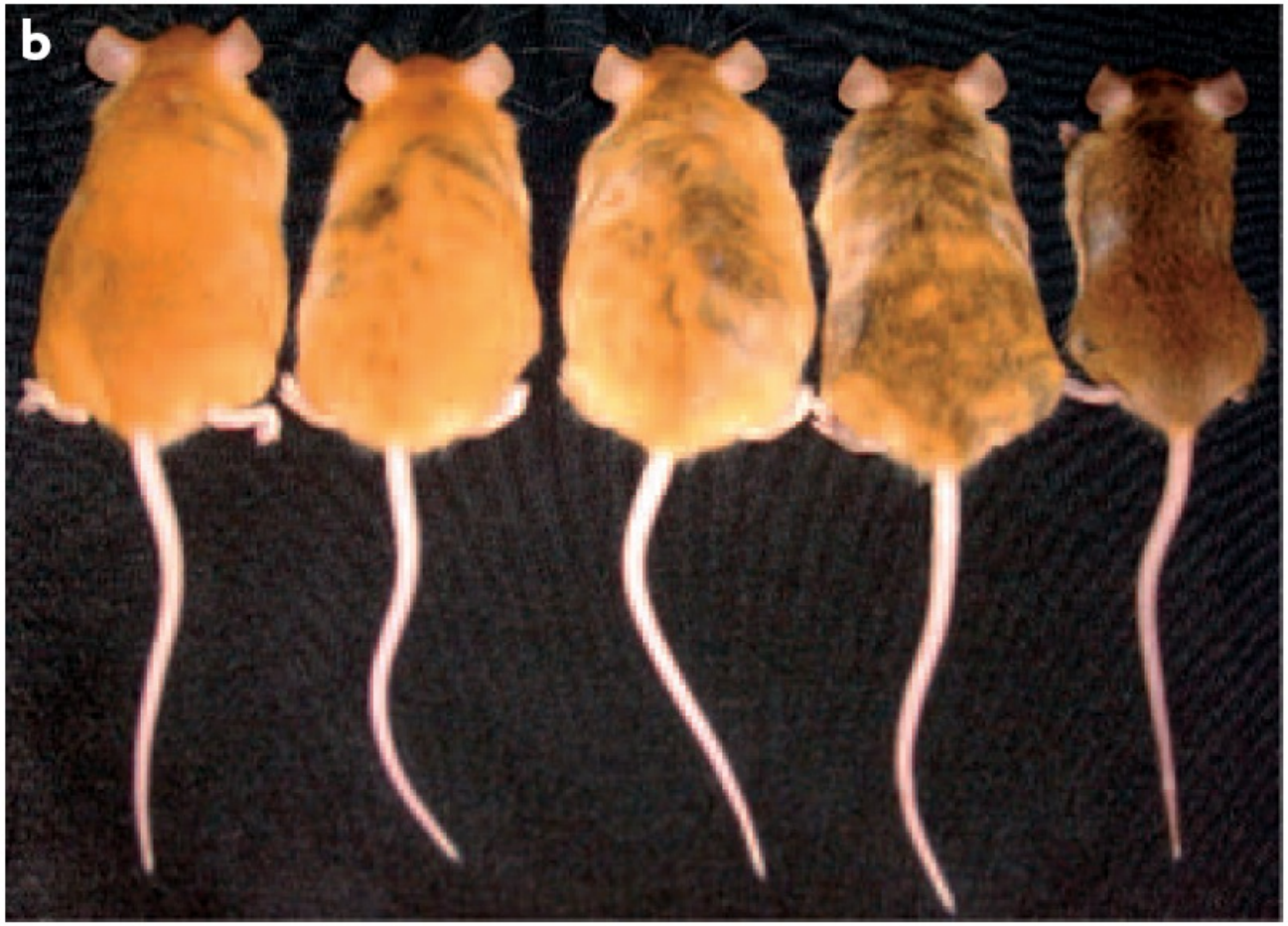
Common Disease Genetic and Epigenetic Hypothesis





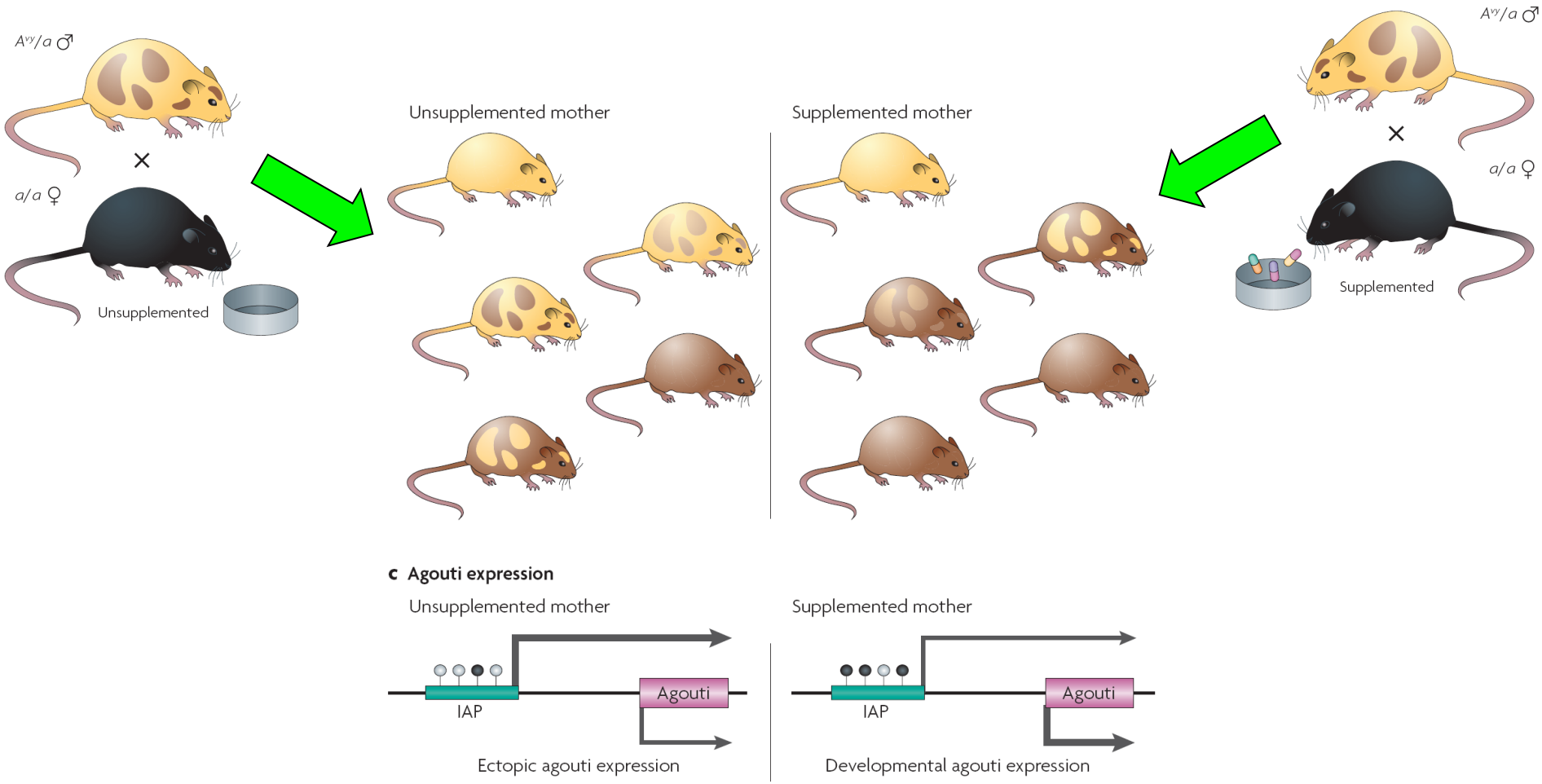
Jirtle et al. Nat Rev Gen 2007; 8: 253-62

Common Disease Genetic and Epigenetic Hypothesis



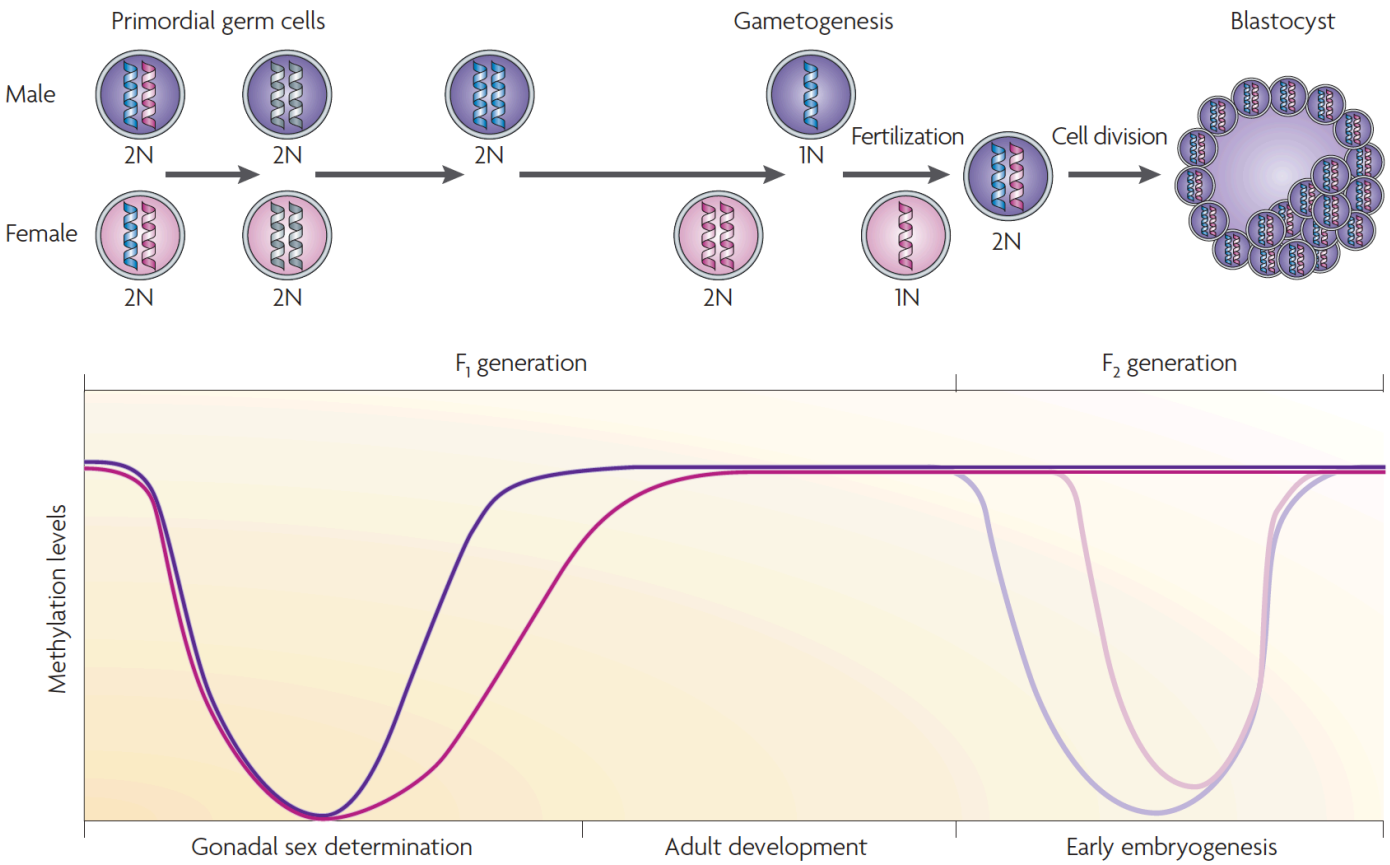
Kaminsky et al. Nat Gen 2009; 41: 240-5

Complex Traits and Epigenetics



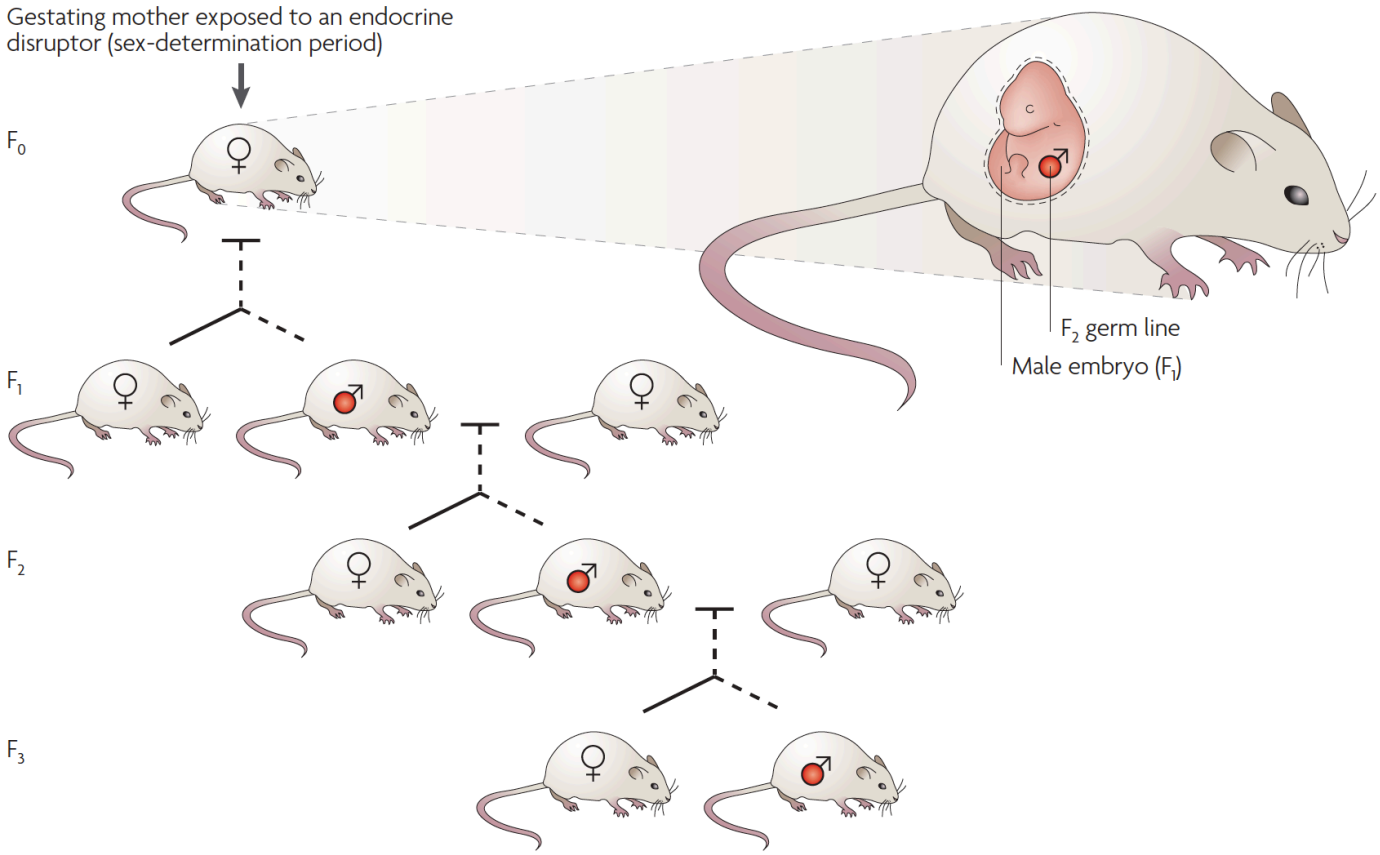
Kaminsky et al. Nat Gen 2009; 41: 240-5

Complex Traits and Epigenetics



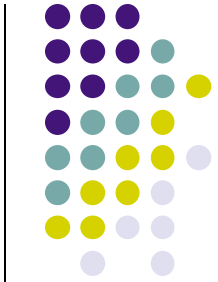
Kaminsky et al. Nat Gen 2009; 41: 240-5

Complex Traits and Epigenetics



Kaminsky et al. Nat Gen 2009; 41: 240-5

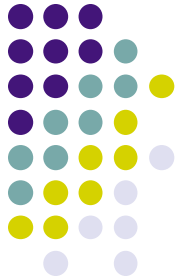
Complex Traits and Epigenetics



- Is this relevant to human biology?
  - In a population of uremic patients, hypomethylation was associated with hyperhomocysteinemia
  - Folate therapy restored DNA methylation to normal levels and corrected the patterns of gene expression

Ingrosso et al., Lancet 2003 May 17;361(9370):1693-9

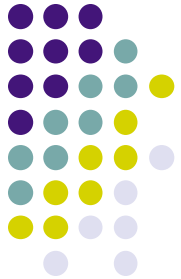
Complex Traits and Epigenetics



- Epigenic programming by maternal behavior
  - Nurturing behavior of female mice is passed from generation-to-generation
  - Not germline inherited but passed on to the offspring directly from the mother during the first week of postnatal life
  - Nurturing behavior in the first week of life is associated with lifelong reduced fearfulness and HPA-axis response to stress

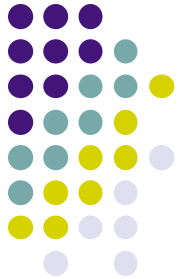
Weaver et al., Nat Neurosci. 2004 Aug;7(8):847-54.

Complex Traits and Epigenetics



- Maternal programming effects involve DNA methylation and histone modifications of a TF binding motif present at the brain-specific GR gene locus
- Experimentally reversible
- Shows that early postnatal life experiences can modify behavior by altering epigenome

Complex Traits and Epigenetics



But is this relevant in Humans?

nature

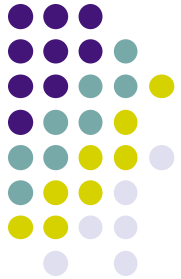
Vol 462|17 December 2009|doi:10.1038/nature08625

ARTICLES

Parental origin of sequence variants associated with complex diseases

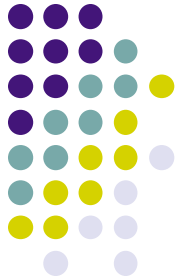
Augustine Kong[1], Valgerdur Steinthorsdottir[1]*, Gisli Masson[1]*, Gudmar Thorleifsson[1]*, Patrick Sulem[1], Soren Besenbacher[1], Aslaug Jonasdottir[1], Asgeir Sigurdsson[1], Kari Th. Kristinsson[1], Adalbjorg Jonasdottir[1], Michael L. Frigge[1], Arnaldur Gylfason[1], Pall I. Olason[1], Sigurjon A. Gudjonsson[1], Sverrir Sverrisson[1], Simon N. Stacey[1], Bardur Sigurgeirsson[2], Kristrun R. Benediktsdottir[3], Helgi Sigurdsson[4], Thorvaldur Jonsson[5], Rafn Benediktsson[6], Jon H. Olafsson[2], Oskar Th. Johannsson[4], Astradur B. Hreidarsson[6], Gunnar Sigurdsson[6], the DIAGRAM Consortium†, Anne C. Ferguson-Smith[7], Daniel F. Gudbjartsson[1], Unnur Thorsteinsdottir[1,8] & Kari Stefansson[1,8]

Complex Traits and Epigenetics



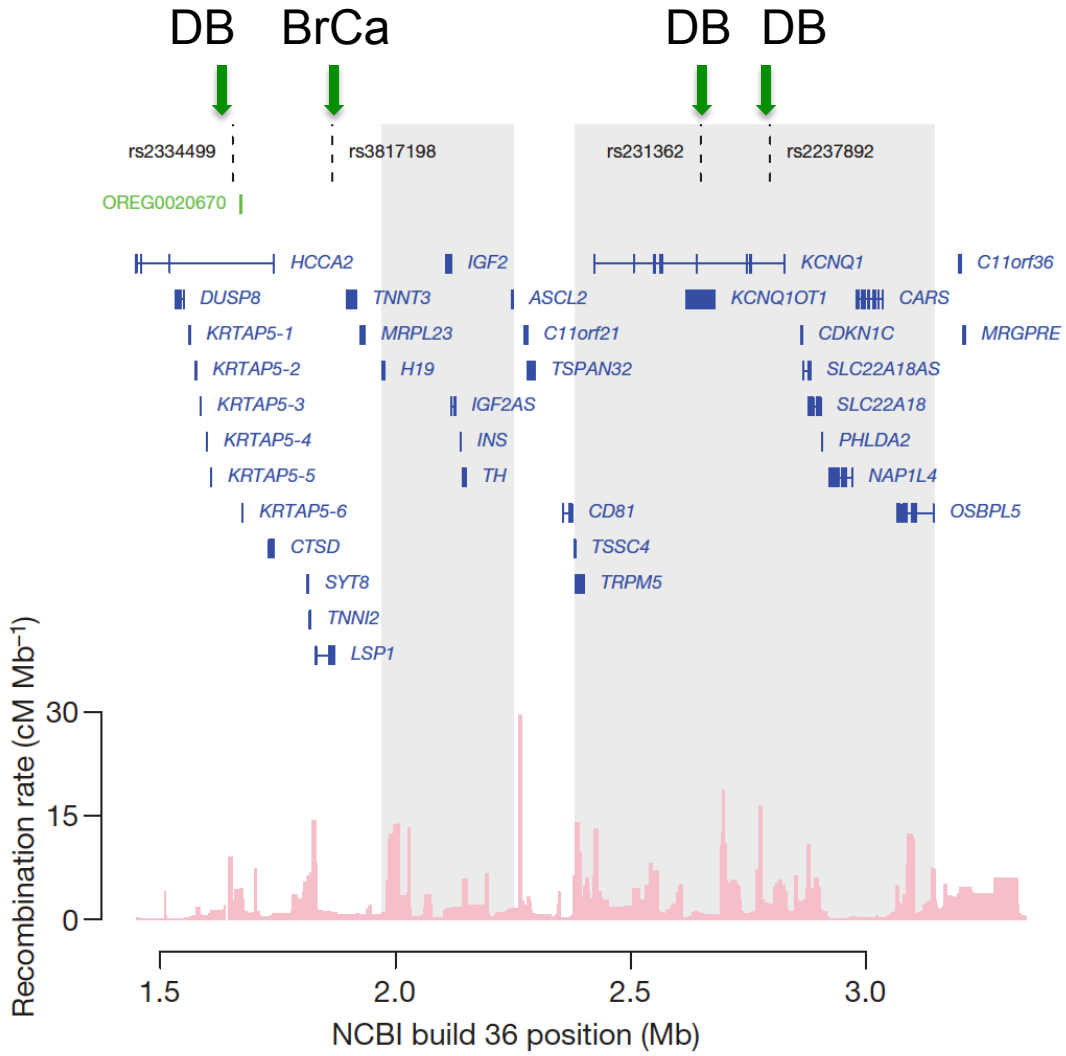
- Focused on SNPs associated with disease within 500 Kb of imprinted genes
- 5 out 7 SNPs examined showed significant prental-origin-specific associations
  - 1 Breast cancer
  - 1 Basal-cell carcinoma
  - 3 Diabetes
- These SNPs are located at 7q32 and 11p15

Complex Traits and Epigenetics



- One novel association with diabetes
  - SNP rs2334499 confers risk when paternally inherited and protective when maternally
  - Seems to decrease methylation at a differentially methylated CTCF-binding site
  - Would not have been detected without taking into account parental origin

Complex Traits and Epigenetics



Epigenetics and Obesity

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

Katherine J Dick, Christopher P Nelson, Loukia Tsaprouni, Johanna K Sandling, Dylan Aïssi, Simone Wahl, Eshwar Meduri, Pierre-Emmanuel Morange, France Gagnon, Harald 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-Alexandre Trégouët, Panos Deloukas, Nilesh J Samani

Lancet 2014; 383: 1990–98

Published Online March 13, 2014 http://dx.doi.org/10.1016/S0140-6736(13)62674-4

Summary

Background Obesity is a major health problem that is determined by interactions between lifestyle and environmental and genetic factors. Although associations between several genetic variants and body-mass index (BMI) have been identified, little is known about epigenetic changes related to BMI. We undertook a genome-wide analysis of methylation at CpG sites in relation to BMI.

Epigenetics and Obesity

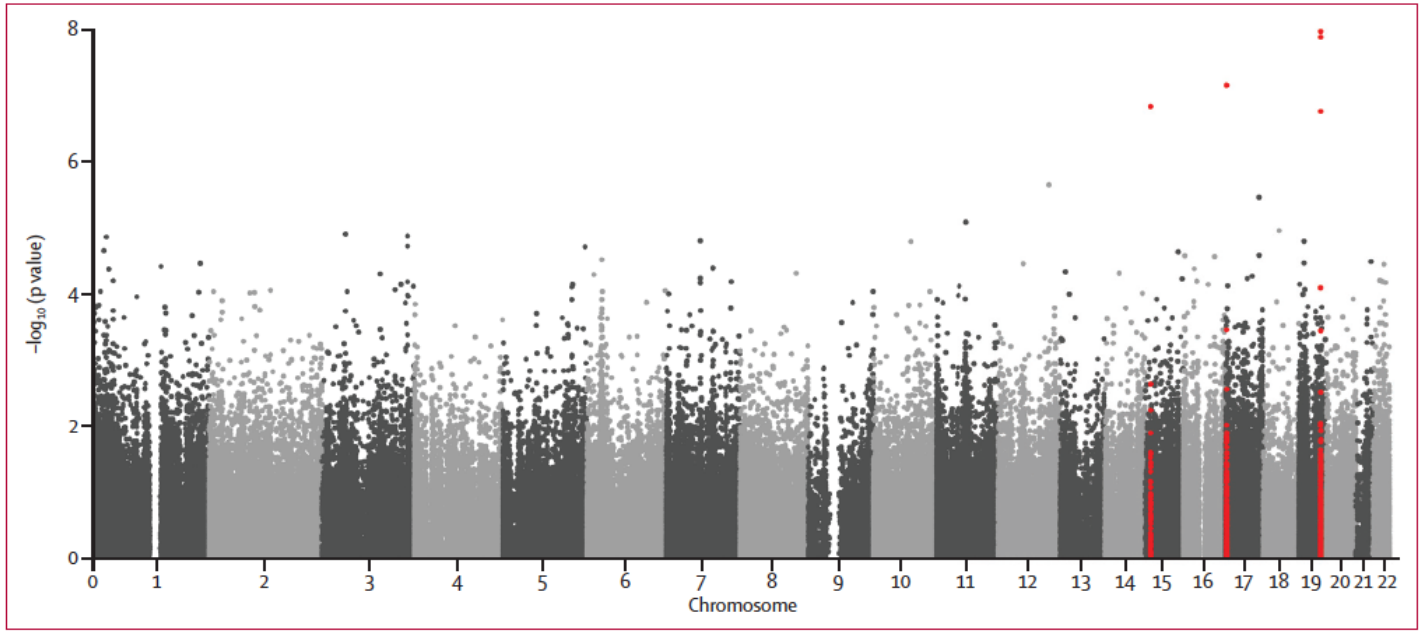


Figure 1: **Manhattan plot showing the distribution of p values of the association of methylation probes with body-mass index in the discovery cohort**
The red dots indicate probes that fall within *KLF13* (chromosome 15), *CLUH* (chromosome 17), and *HIF3A* (chromosome 19).

	Position	Discovery cohort (Cardiogenics)		Primary replication cohort (MARTHA)		Second replication cohort (KORA)	
		p value*	Percentage change in BMI (95% CI)†	p value	Percentage change in BMI†	p value	Percentage change in BMI†
cg22891070	46801642	$4{\cdot}00\times10^{-8}$	3·6% (2·4–4·9)	$3{\cdot}65\times10^{-4}$	2·7% (1·2–4·2)	$6{\cdot}69\times10^{-3}$	0·8% (0·2–1·4)
cg27146050	46801557	$4{\cdot}82\times10^{-8}$	7·8% (5·1–10·4)	$5{\cdot}09\times10^{-3}$	6·2% (1·8–10·4)	$2{\cdot}18\times10^{-3}$	2·1% (0·7–3·4)
cg16672562	46801672	$5{\cdot}36\times10^{-7}$	3·2% (2·0–4·4)	$3{\cdot}47\times10^{-3}$	2·1% (0·7–3·5)	0·011	0·7% (0·2–1·3)

The significance threshold after Bonferroni correction for multiple testing in the primary replication cohort is 0·01 and in the second replication cohort is 0·016. BMI=body-mass index. *λ corrected. †The β coefficients from the association analysis have been converted into percentage change in BMI for every 0·1 unit increase in methylation β value.

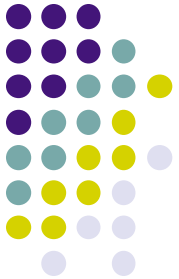
Table 2: **Association between methylation at sites in *HIF3A* on chromosome 19 in whole-blood DNA and BMI in the discovery and replication cohorts**

Epigenetics and Obesity

	Adipose tissue (n=635)		Skin (n=395)	
	p value	Percentage change in BMI*	p value	Percentage change in BMI*
cg22891070	$1{\cdot}72\times10^{-5}$	6·2 (3·4 to 9·0)	0·882	−0·25 (−3·6 to 3·0)
cg27146050	$9{\cdot}27\times10^{-7}$	11·9 (7·2 to 16·7)	0·011	−7·0 (−12·4 to −1·7)
cg16672562	$5{\cdot}01\times10^{-6}$	7·9 (4·5 to 11·2)	0·862	−0·36 (−4·3 to 3·5)

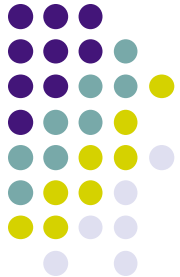
Data in parentheses are 95% CIs. BMI=body-mass index. *The β coefficients from the association analysis have been converted into percentage change in BMI for every 0·1 unit increase in methylation β value.

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



Future directions

Epigenetics Cartography

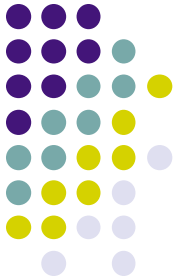


- International Human Epigenome Consortium
  - Reference maps of human epigenomes
  - Aims to decipher 1000 epigenomes
  - Histone modifications and DNA methylation
  - Why is this important?

- ENCODE

www.ihec-epigenomes.org

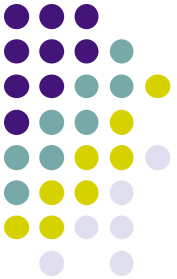
Epigenetics and Ethics



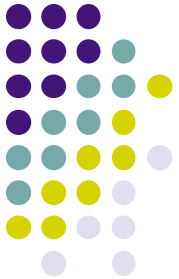
- Environmental Justice
  - “Epigenetics provides a new window for understanding and possibly addressing the co-morbidities associated with disparate environmental exposures”
- Intergenerational effects and equity
  - “Each generation has an obligation to its descendant not to damage the genomes and epigenomes of future generations, such as through exposure to environmental hazards.”
- Privacy and confidentiality issues

Rothstein et al. Nat Rev Gen AOP

Bringing the Methylome to Medicine



- Diagnostic testing
  - Cancer biomarker
  - Marker of environmental exposure
  - …
- Therapeutic tool
  - Agents that modify the epigenome globally
  - “Conventional” medicinal therapy targeting biochemical pathways that are disturbed epigenetically in disease



Thanks!