Epigenetics in clinical medicine; learning and taming of the functional genome

Tomas Ekström, Ph.D.
Center for Molecular Medicine
(CMM)
Karolinska Institutet/Hospital

Karolinska Institutet

IMU Nov. 2011

Outline

- Principles of epigenetics
- Experimental project, viral impact on epigenetic mechanisms
- Clinically oriented approaches using human subjects/patient material to assess epigenetics for phenotype and risk

Epigenetics is mitotic and/or meiotic heritable property in gene function which…

…can not be explained by the DNA sequence.

The conductor/operating system of the genome

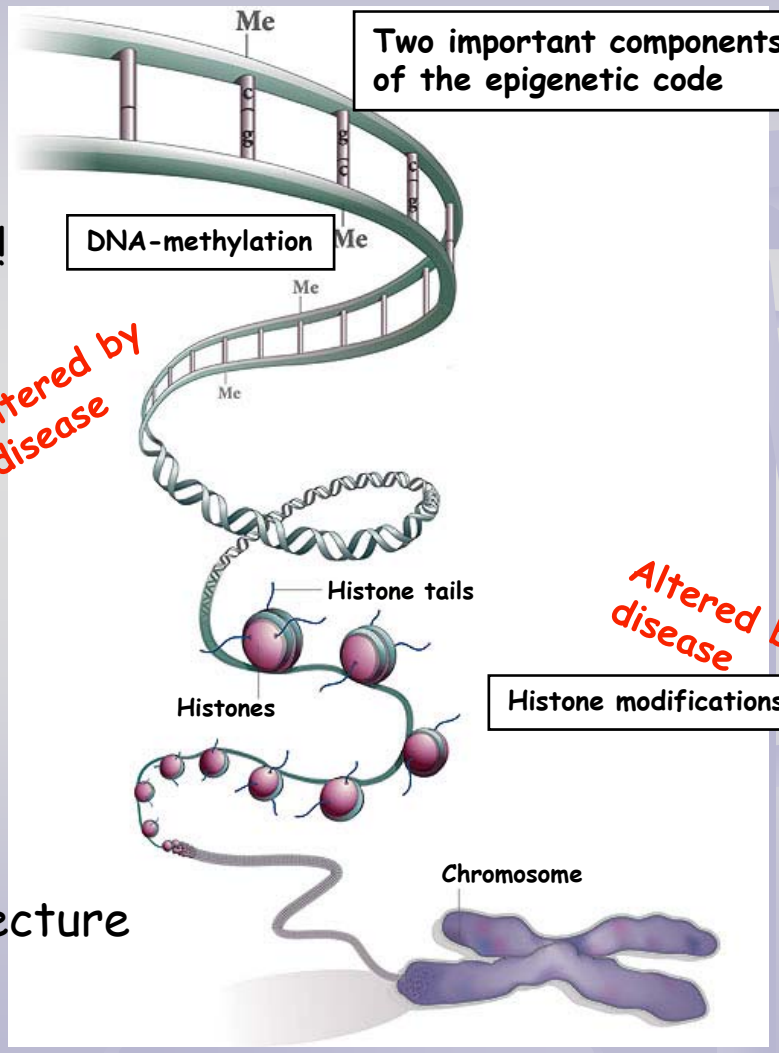
Karolinska Institutet

The epigenetic code

The epigenetic code
is unique for each cell type!

...and for each disease

Controls the cell's properties and cell memory



Gene expression programs
› Cellular phenotype/ architecture

Karolinska Institutet

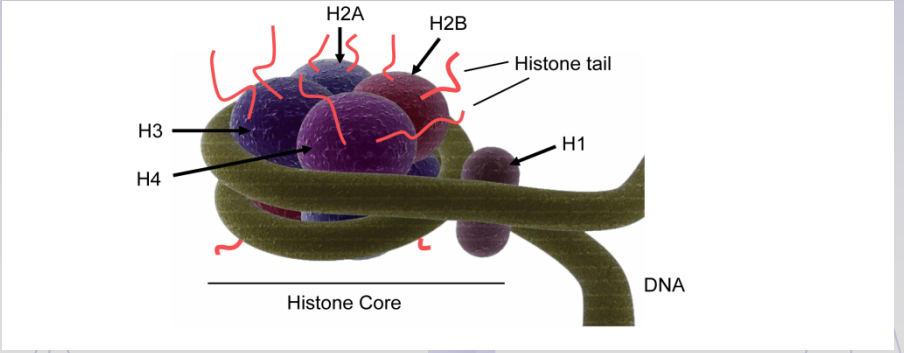
The epigenetic state

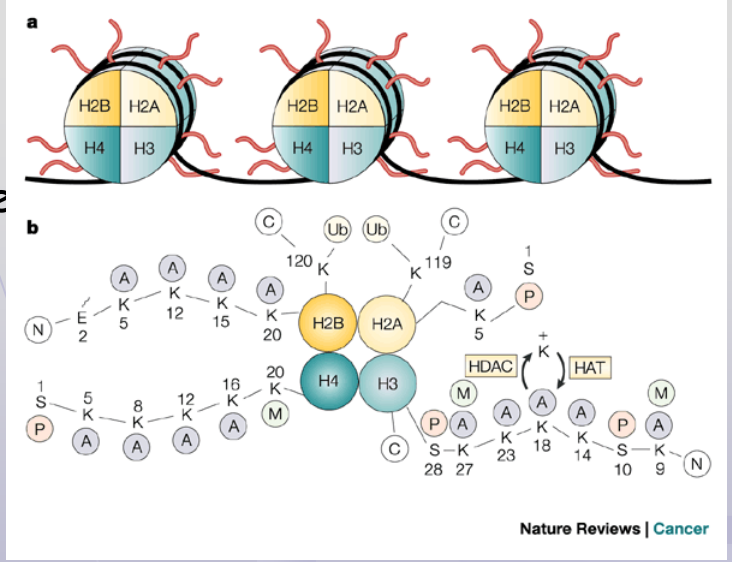
- Posttranslational histone modifications of nucleosomal histone N-terminal tails
  The "Histone Code"

and

- DNA modifications
  Methylation of Cytosines in CpG dinucleotide
  De Novo and Maintenance methylation

Cross-talk





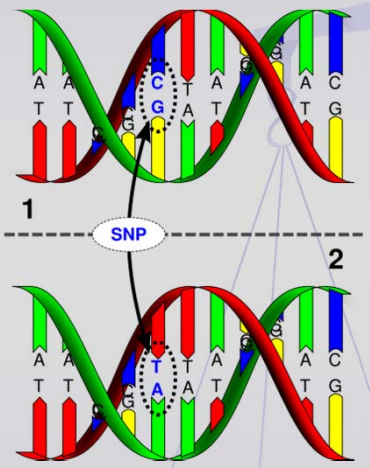
Environment

- Diet
- Physical activity
- Intrauterine environment
- Infection
- Sleep debt
- Endocrine disrupting chemicals
- Pharmaceutical side effects
- Ambient temperature

Direct toxic or mutagenic effects

Genotype

- Gene polymorphisms
- Spontaneous mutations



Variable metabolic enzyme activities, structural proteins

Stochasticity

Epigenotype

- DNA-methylation
- Histone modifications
- Small RNAs
- Chromatin-modulating proteins

Altered gene regulation, ageing, disease

Phenotypic plasticity

Phenotype

IMU Nov. 2011

Epigenetic changes leading to new cell identities

Permissiveness!!!
Driving differentiation
Preventing dediff.

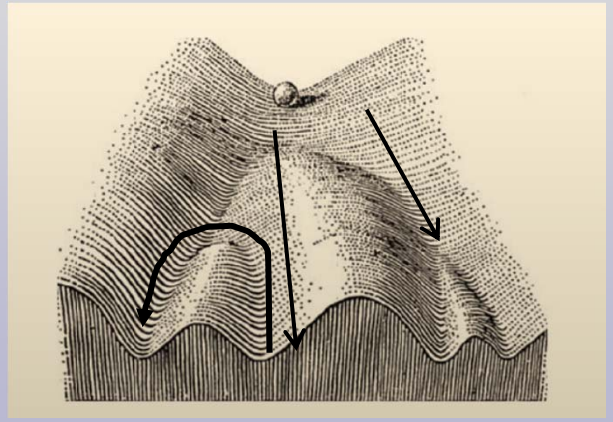
Totipotent stem cell

Celltype 1, e.g. liver cell

Cell type 2, e.g. skin cell

Cell type 3, e.g. nerve cell

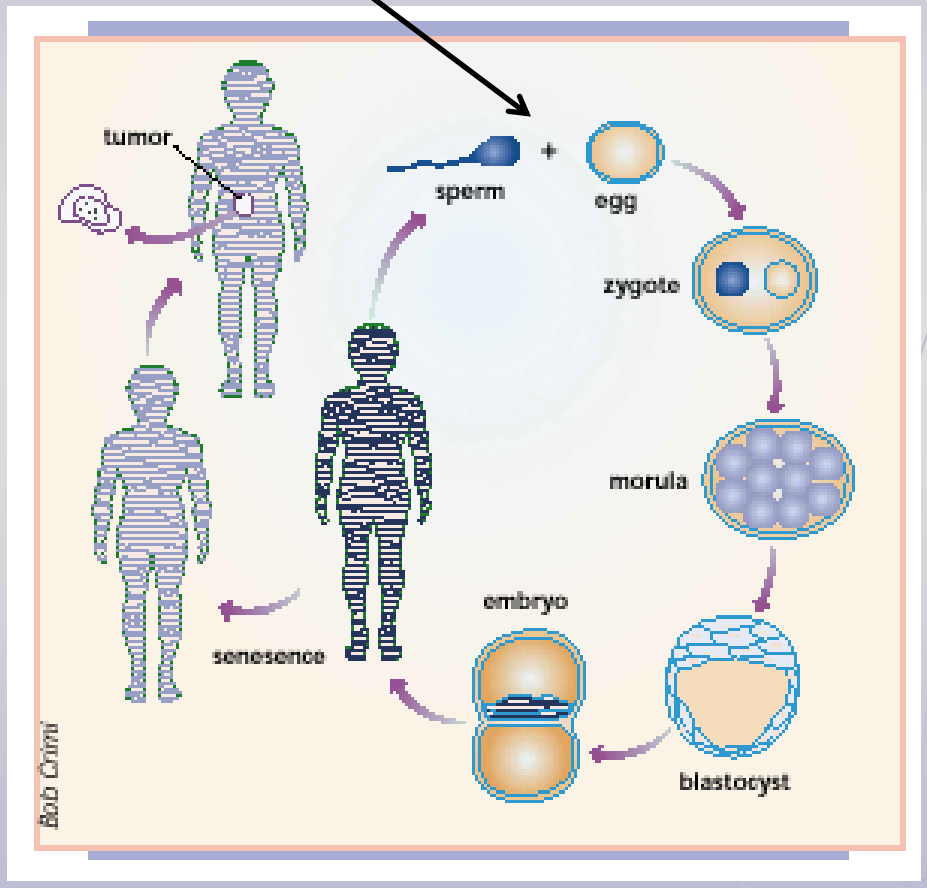
Cell type 4, e.g. muscle cell



Waddington's epigenetic landscape (1957)

The epigenome has a life cycle, the genome does not

Epigenetic reset and reprogramming in germ line

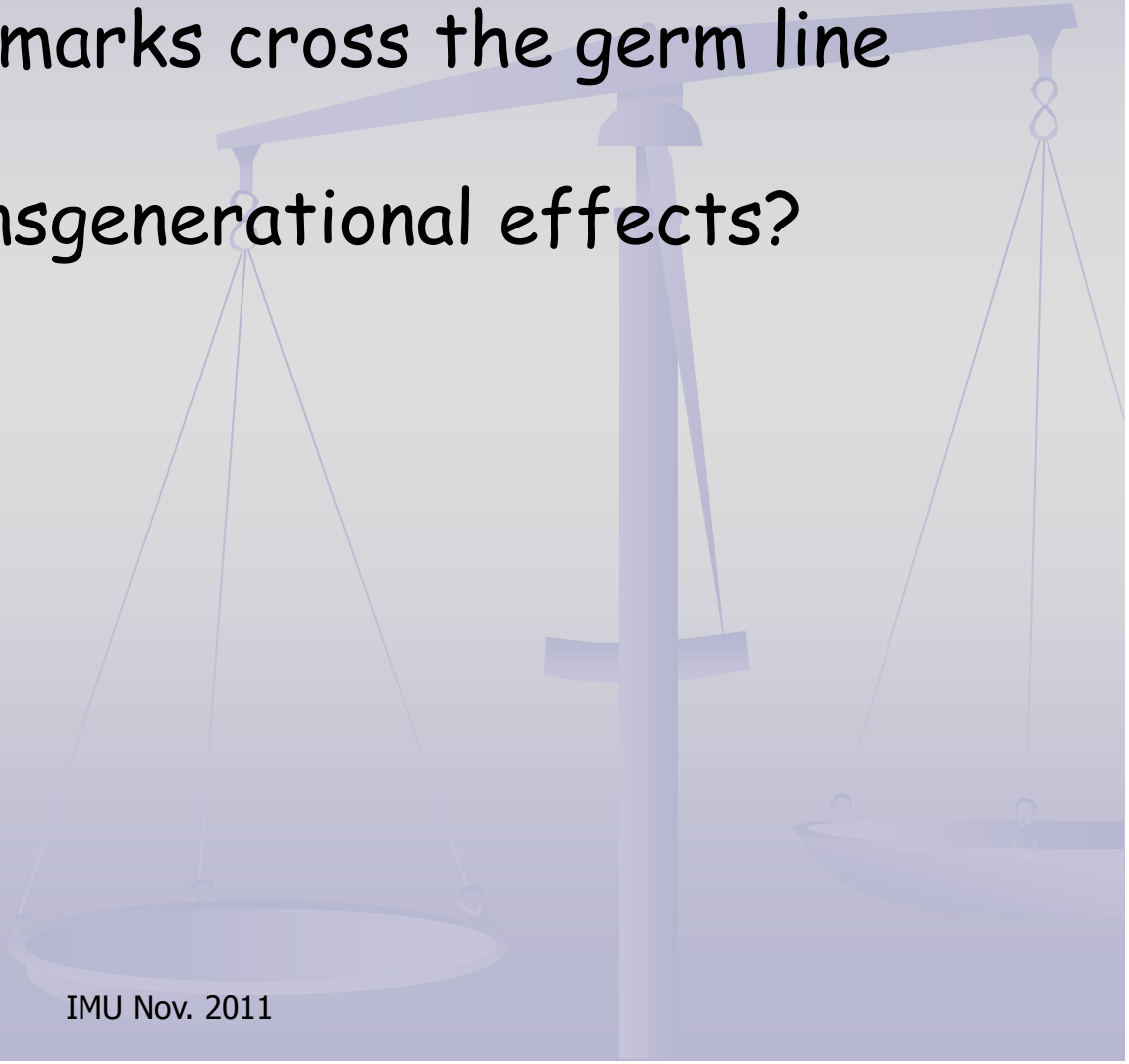


Epigenetic marks/landscapes distinguish:

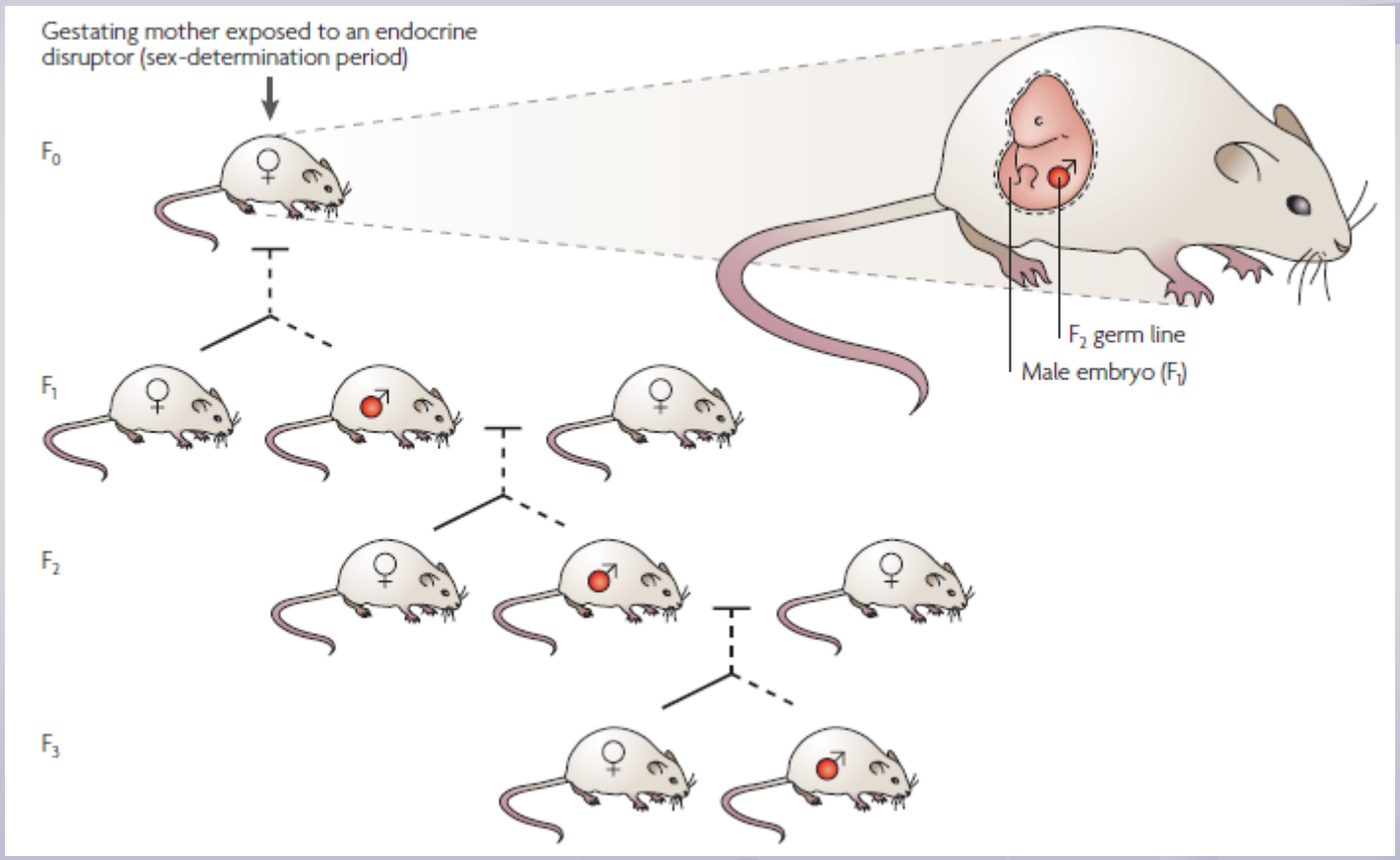
- Stem cells
- Tissue types
- Ageing
- Cancer
- Cardiovascular disease
- Renal disease
- Diabetes
- Brain disorders
  - Depression
  - Alzheimers
  - Schizophrenia
  - Stress
- Environmental impact

Can epigenetic marks cross the germ line

and give transgenerational effects?



Transgenerational (to F3) effects on epigenetic modification by the antiandrogenic Vinclozolin

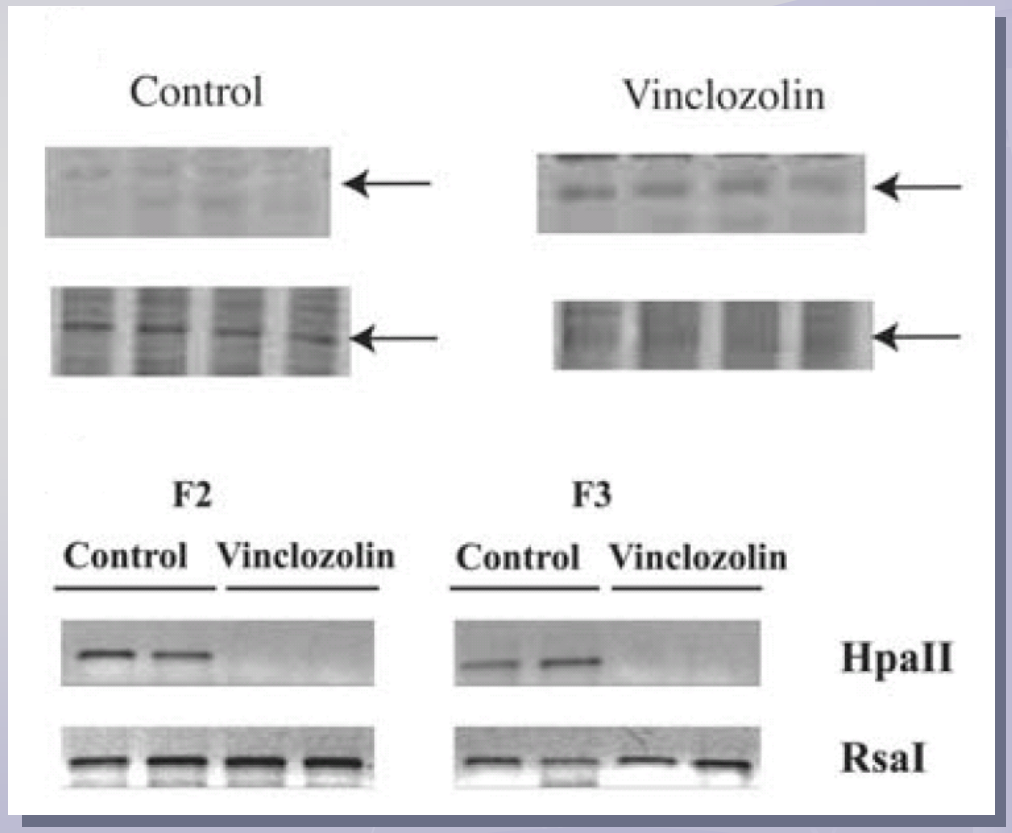


Anway et al. Endocrinol. 2006

Transgenerational (to F3) effects on epigenetic modification by the antiandrogenic Vinclozolin

Alteration in DNA-methylation

Genomewide DNA-methylation changes



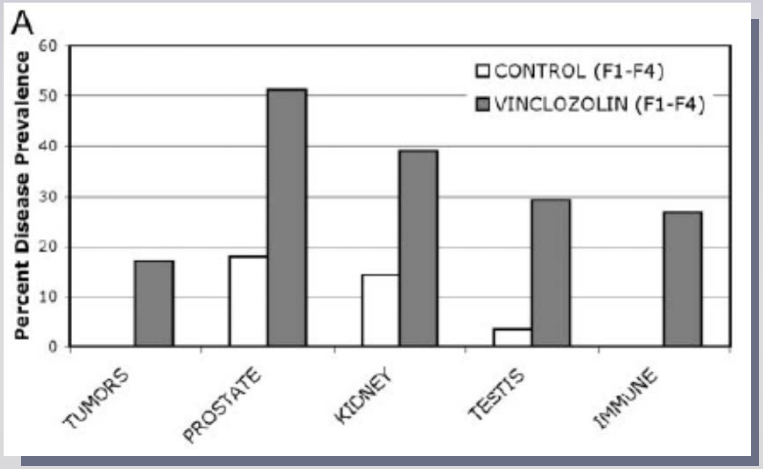
DNA-methylation changes in the cytokine-inducible SH2 protein gene

Karolinska Institutet

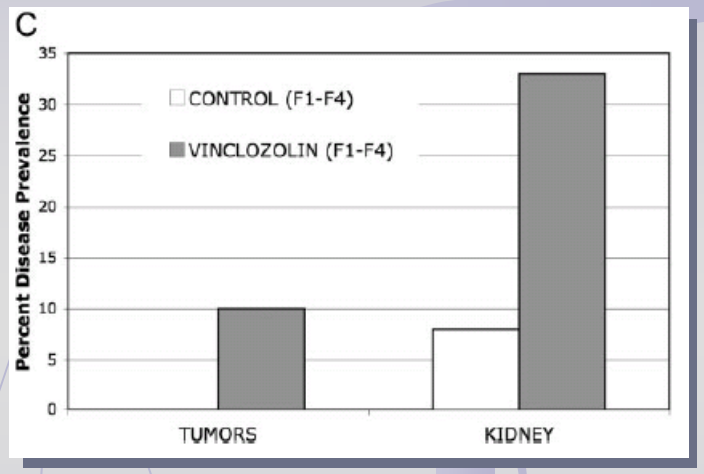
From Anway et al., (2005) Science, 308,1466-1469

Adult disease states or abnormalities in vinclozolin and control generation (F1–F4) animals.

Males



Females



Karolinska Institutet

Anway et al. Endocrinol. 2007

The effect of grandparental food supply at different times in their early life, on the mortality rate of their grandsons

good supply = red squares
poor supply = green sq

Does resetting of the epigenetic marks fail?

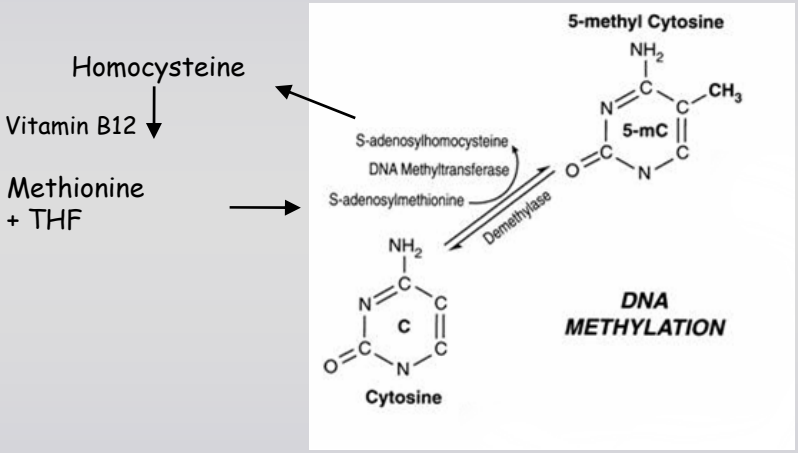
Karolinska Institutet

Marcus E Pembrey, Lars Olov Bygren, Gunnar Kaati, Sören Edvinsson, Kate Northstone, Michael Sjöström, Jean Golding and The ALSPAC Study Team

European Journal of Human Genetics (2006) 14, 159–166

Availability of methylation, dietary consequences for clinical epigenetics

Why is this important…?



Affected by Bisphenol A in plastic food containers

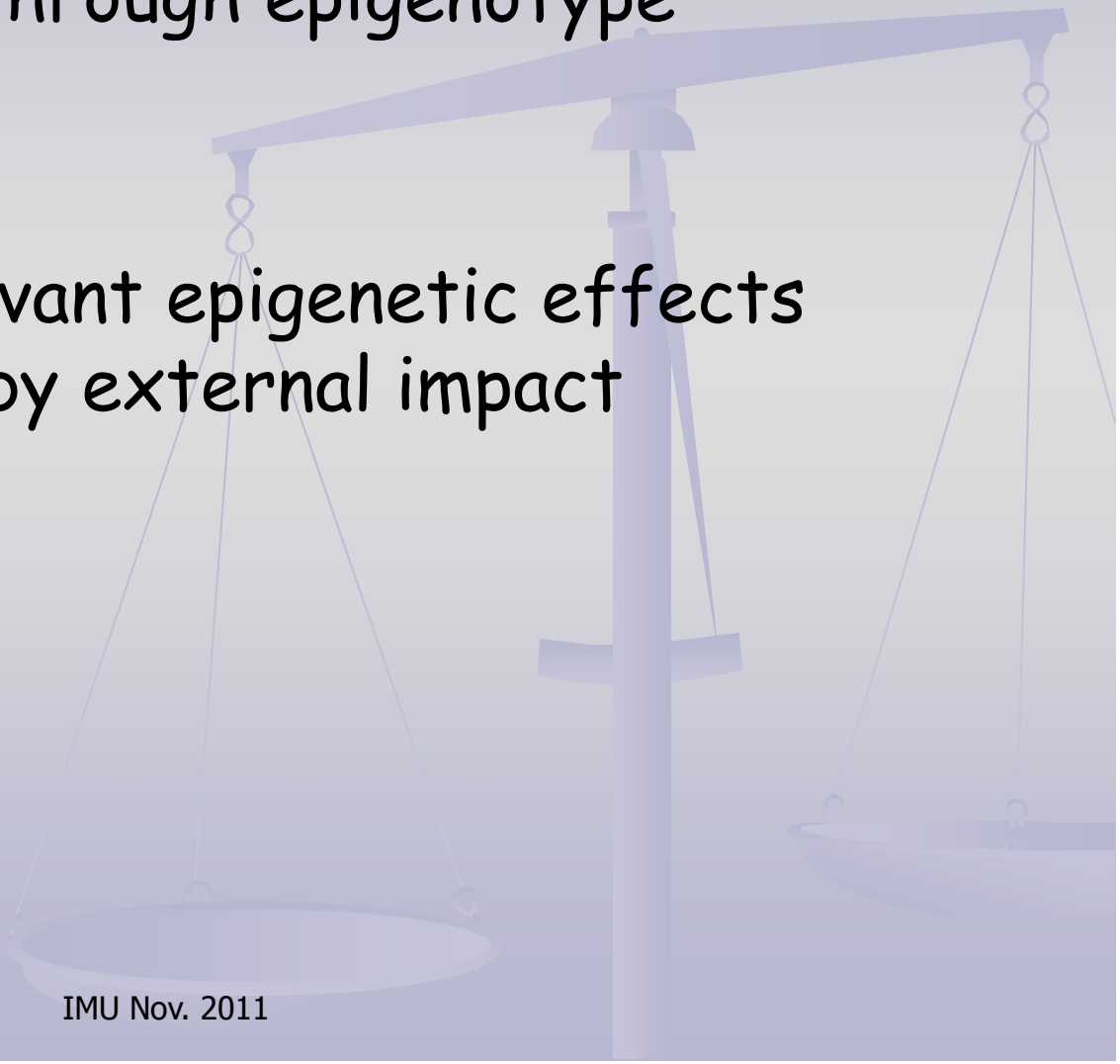
Counteracted by:
Genistein, choline, betaine, folate, Vit.B12

Same genome, *Avy* (viable yellow agouti).
Systemic expression of agouti protein impairs satiety

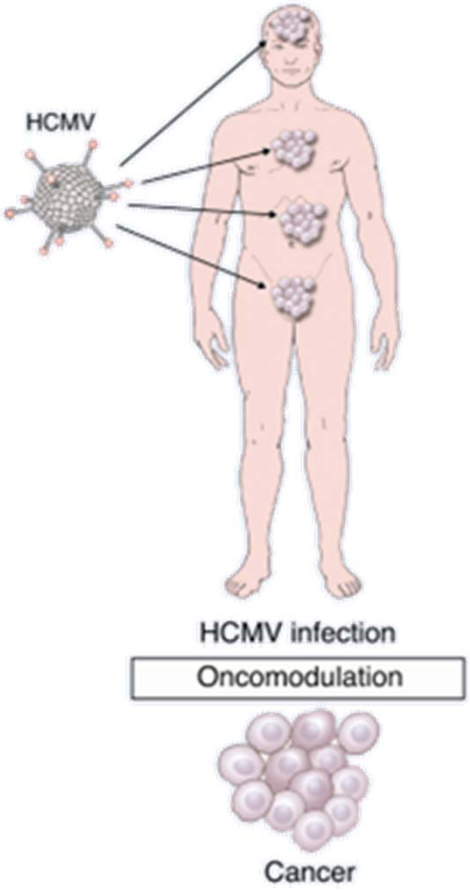
Karolinska Institutet

...because it helps understanding how environment may affect phenotype sensitivity through epigenotype

Clinically relevant epigenetic effects caused by external impact



The potential role of CMV in cancer
Clinical Effects by Epigenotype?



- Frequent presence of genome and antigens of CMV in certain malignant tumors
  - colon cancer
  - glioblastoma multiforme
  - EBV-negative Hodgkin´s lymphoma
  - cervix cancer
  - prostatic carcinoma
  - breast cancer

  - Non-cancer cells in the vicinity of the tumor cells appear to be CMV negative!

Transformation by CMV not known
Epigenetic components involved?

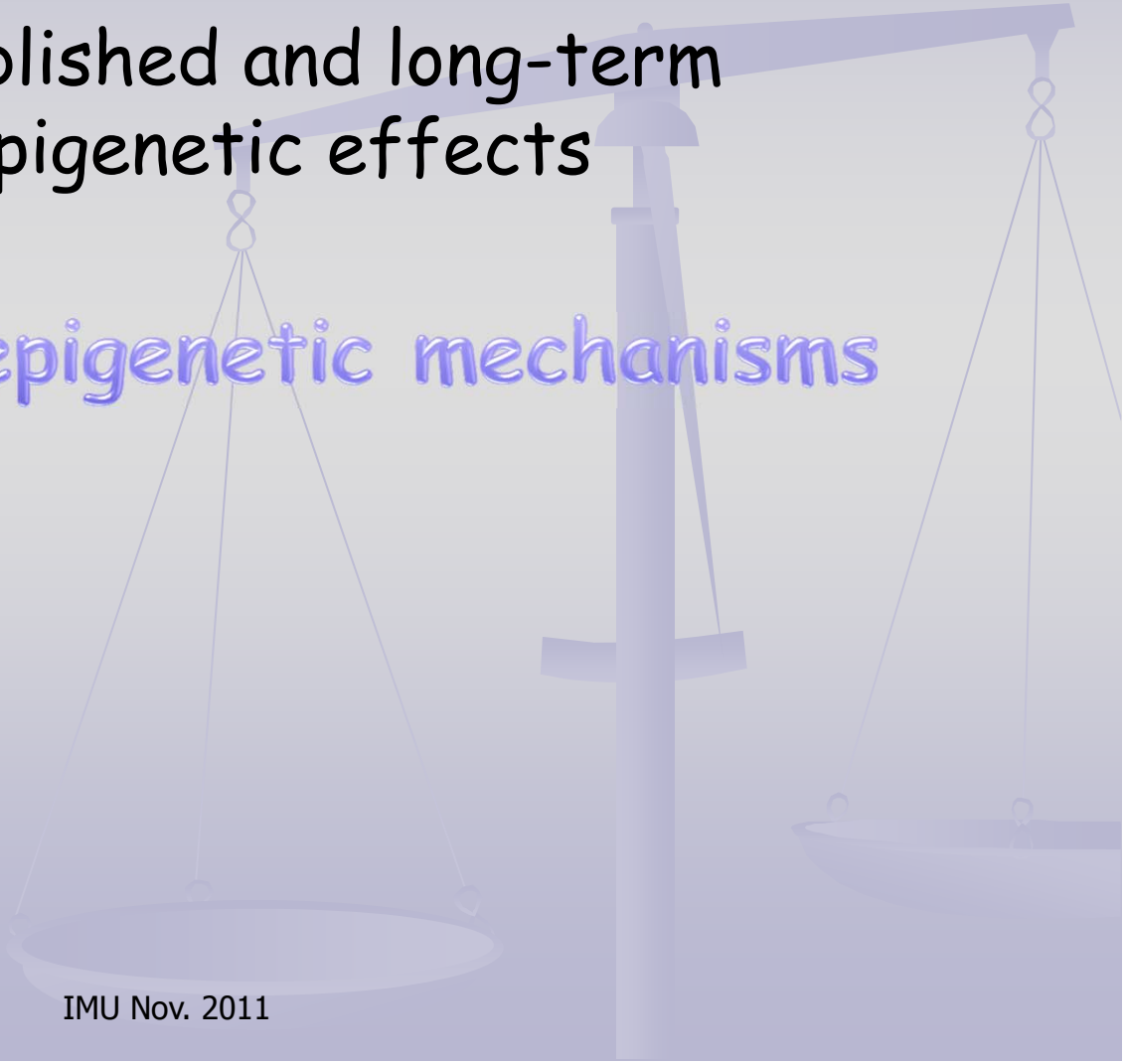
Cytomegalovirus, a common epigenomic abuser?

1. The cellular DNA-methylation state affects infection susceptibility.

2. CMV causes intracellular relocalization of epigenetic modifiers, preventing DNA-methylation in a virus strain-specific manner.

Early established and long-term adult epigenetic effects

Plasticity by epigenetic mechanisms

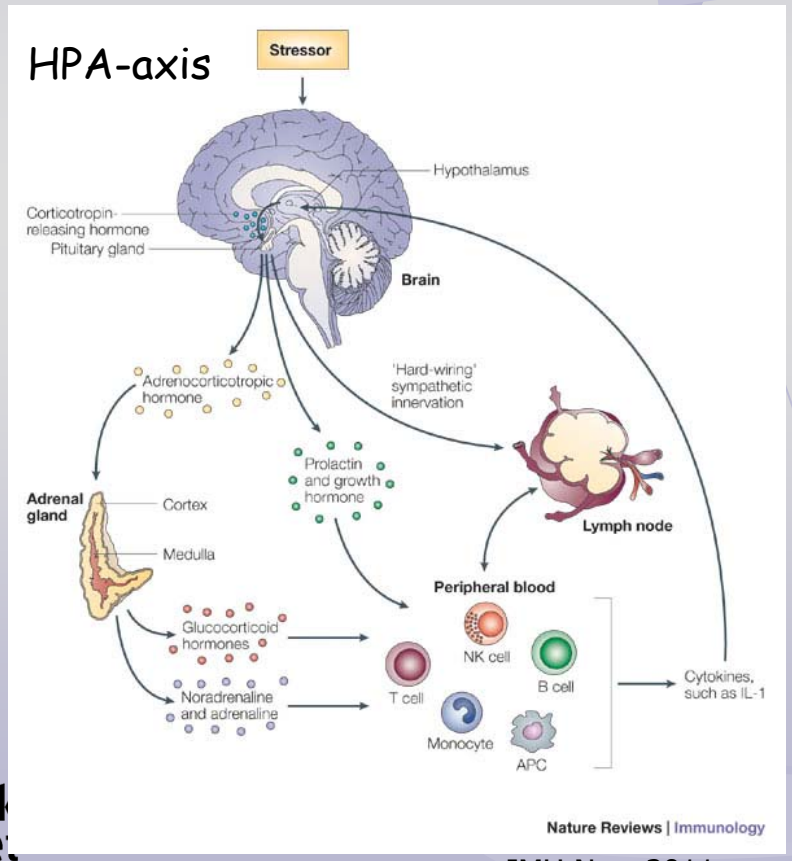


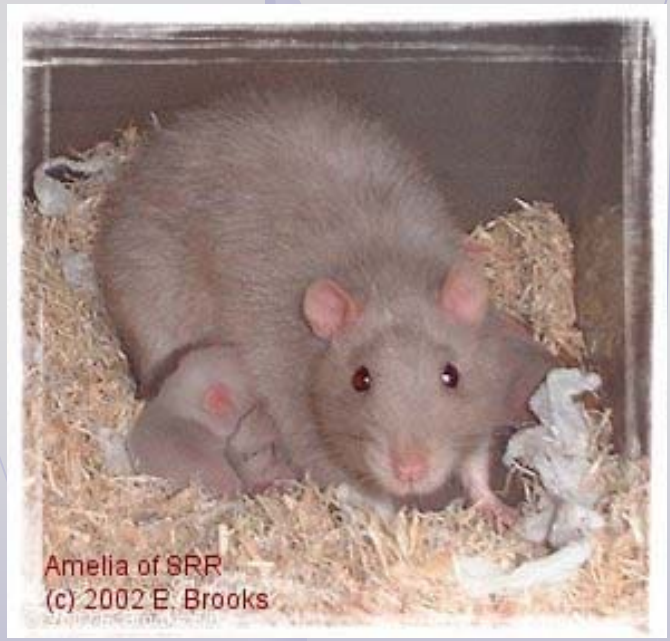
nature neuroscience

Epigenetic programming by maternal behavior

Ian C G Weaver[1,2], Nadia Cervoni[3], Frances A Champagne[1,2], Ana C D'Alessio[3], Shakti Sharma[1], Jonathan R Seckl[4], Sergiy Dymov[3], Moshe Szyf[2,3] & Michael J Meaney[1,2]

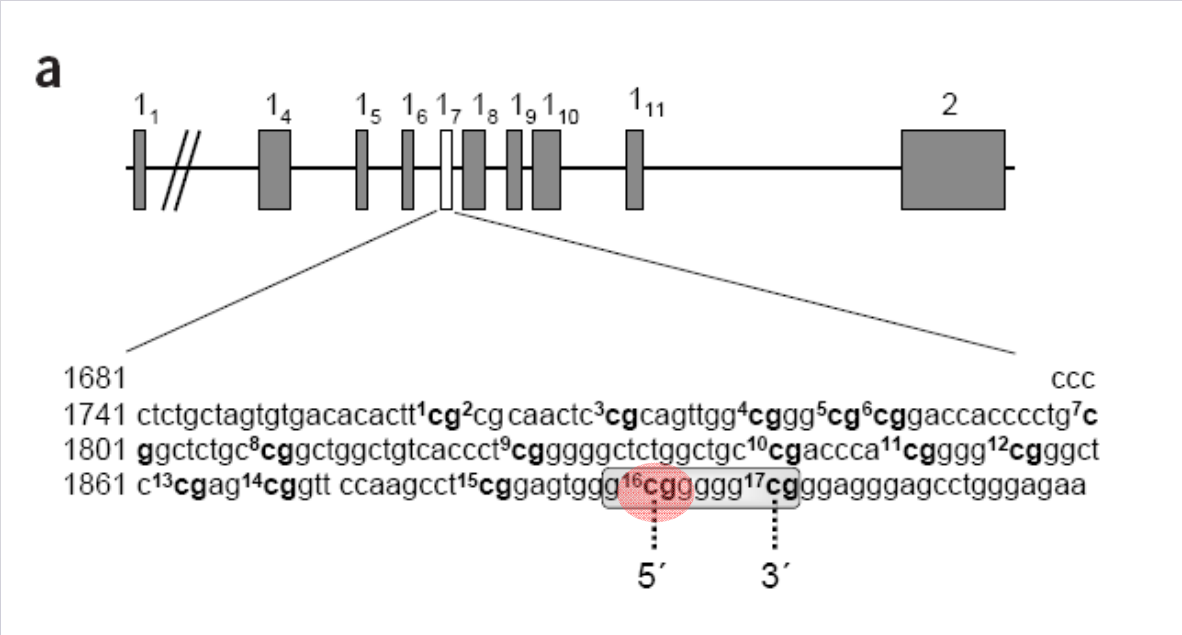
HPA-axis

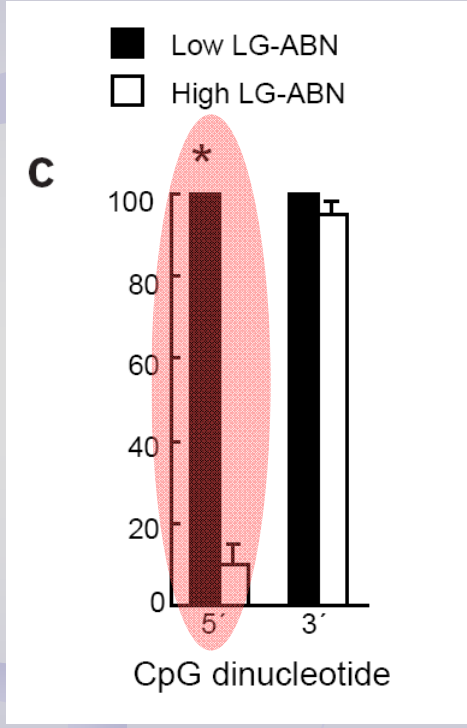




Inheritance of acquired behaviour…?

GR gene in the hippocampus

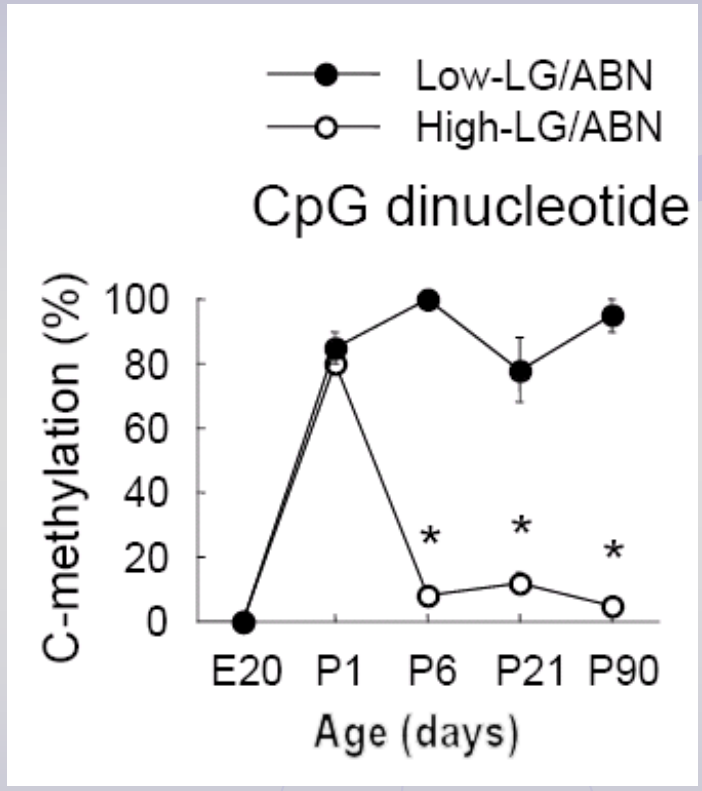




NGFI-A binding site

Weaver et al. Nature Neurosci. 2004

Change in offspring hippocampal epigenetics due to maternal behaviour...?



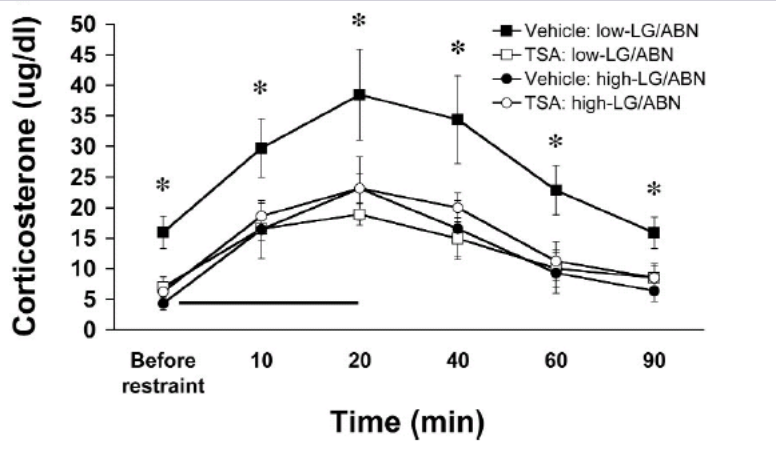
Dynamics of age dependent DNA-methylation in the GR-promoter

Karolinska Institutet

Weaver et al. Nature Neurosci. 2004

...and reversal of maternal effects on stress response by epigenetic drug

Serum cortico-sterone



Remedies for bad mothers...?

Karolinska Institutet

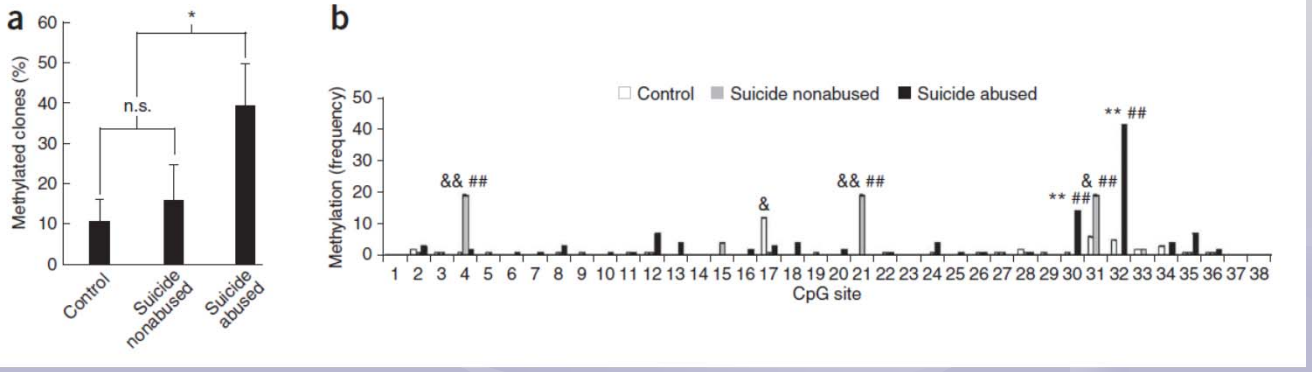
Weaver et al. Nature Neurosci. 2004

How about people??

nature neuroscience

Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse

Patrick O McGowan[1,2], Aya Sasaki[1,2], Ana C D'Alessio[3], Sergiy Dymov[3], Benoit Labonté[1,4], Moshe Szyf[2,3], Gustavo Turecki[1,4] & Michael J Meaney[1,2,5]

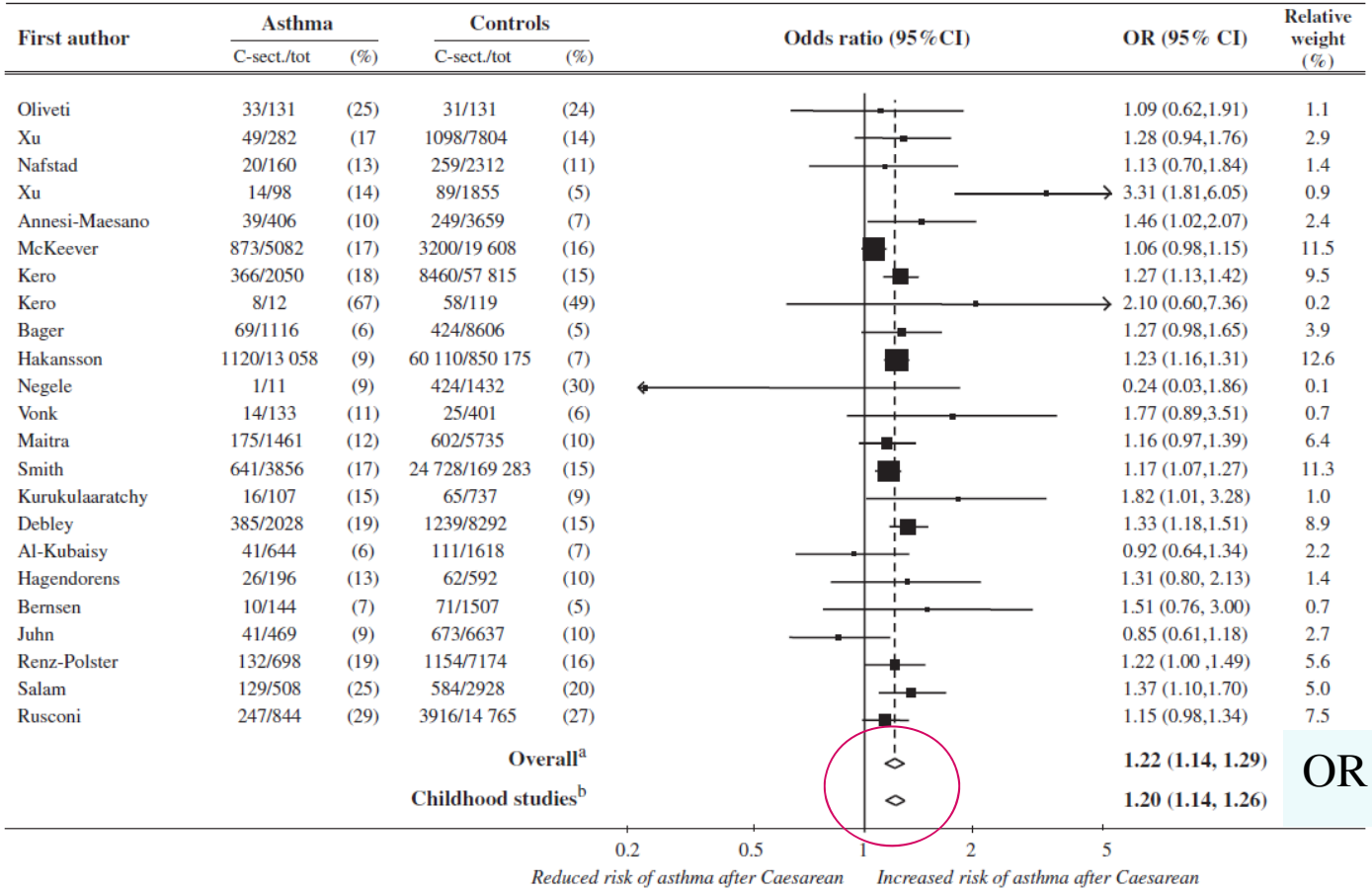


The stress of being born normally (vs. c-section) impacts (positively?) on the global methylome.

Long term effects?

Risk for autoimmunity?

C-section recently associated with increased risk for auto-immune diseases such as asthma…

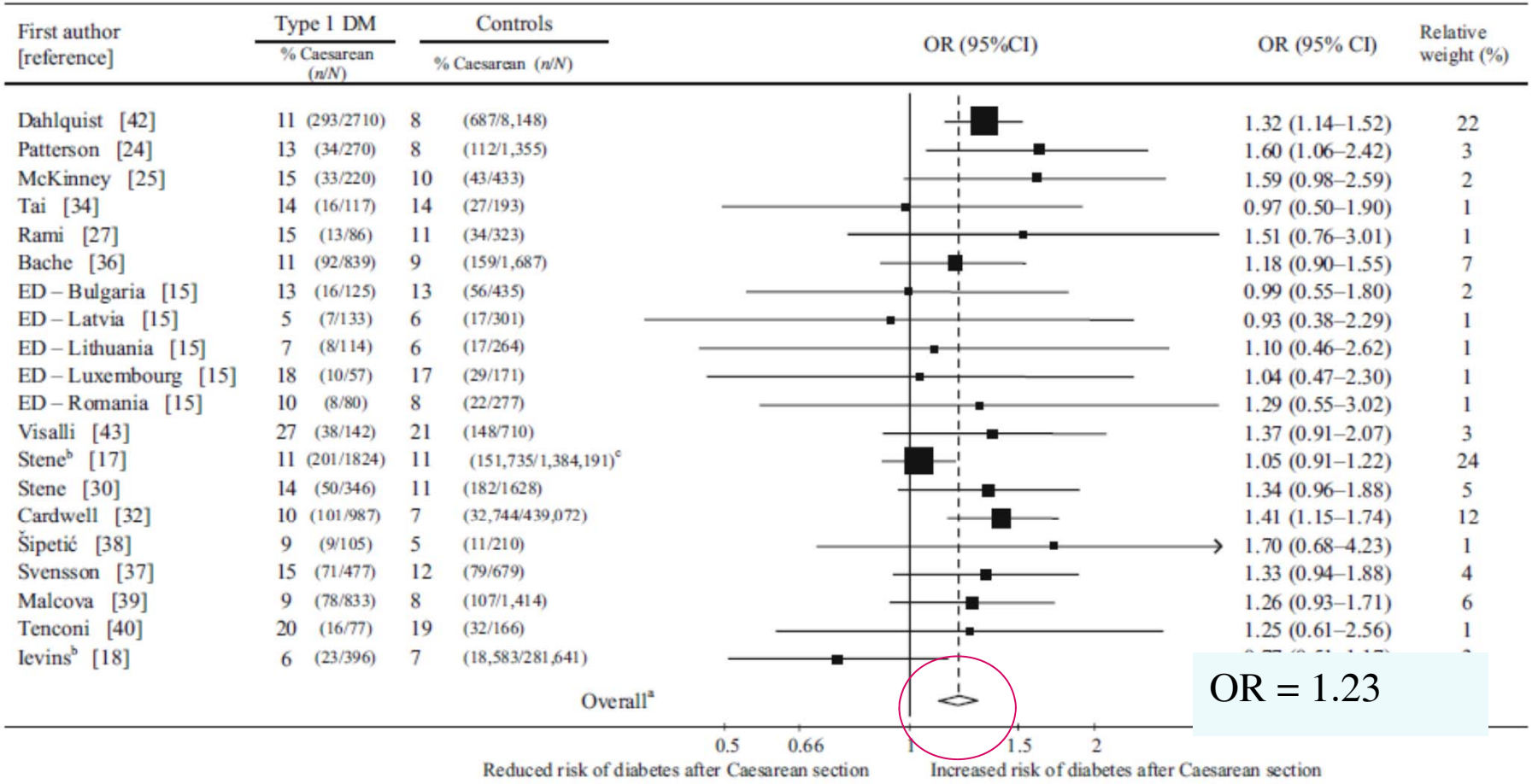


First author	Asthma C-sect./tot	(%)	Controls C-sect./tot	(%)	OR (95% CI)	Relative weight (%)
Oliveti	33/131	(25)	31/131	(24)	1.09 (0.62,1.91)	1.1
Xu	49/282	(17	1098/7804	(14)	1.28 (0.94,1.76)	2.9
Nafstad	20/160	(13)	259/2312	(11)	1.13 (0.70,1.84)	1.4
Xu	14/98	(14)	89/1855	(5)	3.31 (1.81,6.05)	0.9
Annesi-Maesano	39/406	(10)	249/3659	(7)	1.46 (1.02,2.07)	2.4
McKeever	873/5082	(17)	3200/19 608	(16)	1.06 (0.98,1.15)	11.5
Kero	366/2050	(18)	8460/57 815	(15)	1.27 (1.13,1.42)	9.5
Kero	8/12	(67)	58/119	(49)	2.10 (0.60,7.36)	0.2
Bager	69/1116	(6)	424/8606	(5)	1.27 (0.98,1.65)	3.9
Hakansson	1120/13 058	(9)	60 110/850 175	(7)	1.23 (1.16,1.31)	12.6
Negele	1/11	(9)	424/1432	(30)	0.24 (0.03,1.86)	0.1
Vonk	14/133	(11)	25/401	(6)	1.77 (0.89,3.51)	0.7
Maitra	175/1461	(12)	602/5735	(10)	1.16 (0.97,1.39)	6.4
Smith	641/3856	(17)	24 728/169 283	(15)	1.17 (1.07,1.27)	11.3
Kurukulaaratchy	16/107	(15)	65/737	(9)	1.82 (1.01, 3.28)	1.0
Debley	385/2028	(19)	1239/8292	(15)	1.33 (1.18,1.51)	8.9
Al-Kubaisy	41/644	(6)	111/1618	(7)	0.92 (0.64,1.34)	2.2
Hagendorens	26/196	(13)	62/592	(10)	1.31 (0.80, 2.13)	1.4
Bernsen	10/144	(7)	71/1507	(5)	1.51 (0.76, 3.00)	0.7
Juhn	41/469	(9)	673/6637	(10)	0.85 (0.61,1.18)	2.7
Renz-Polster	132/698	(19)	1154/7174	(16)	1.22 (1.00 ,1.49)	5.6
Salam	129/508	(25)	584/2928	(20)	1.37 (1.10,1.70)	5.0
Rusconi	247/844	(29)	3916/14 765	(27)	1.15 (0.98,1.34)	7.5
			Overall[a]		**1.22 (1.14, 1.29)**	
			Childhood studies[b]		**1.20 (1.14, 1.26)**	

OR = 1.22

Thavagnanam S, 2008

..and type 1 diabetes mellitus…

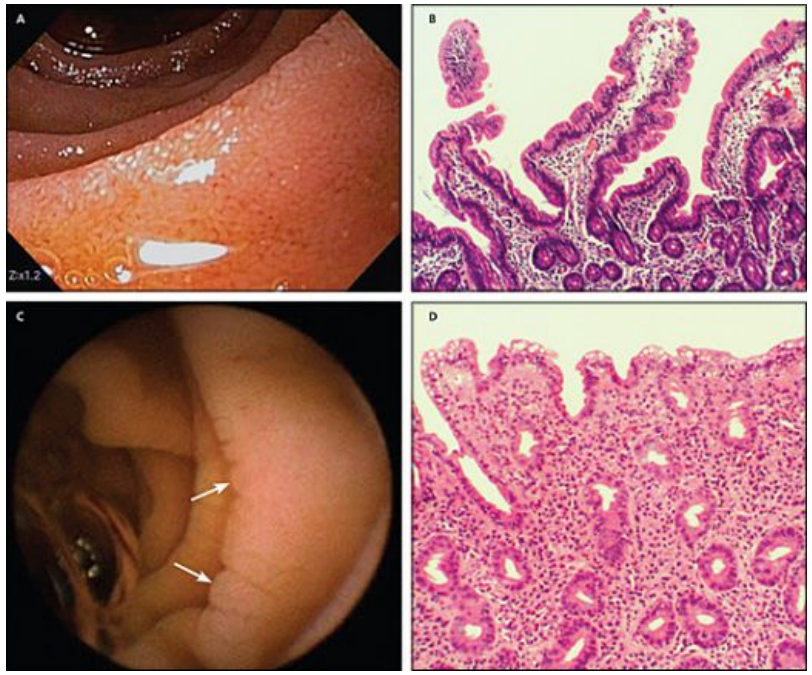


OR = 1.23

Cardwell CR, 2008

..and celiac disease…

OR = 1.8 [95%CI 1.13-2.88]



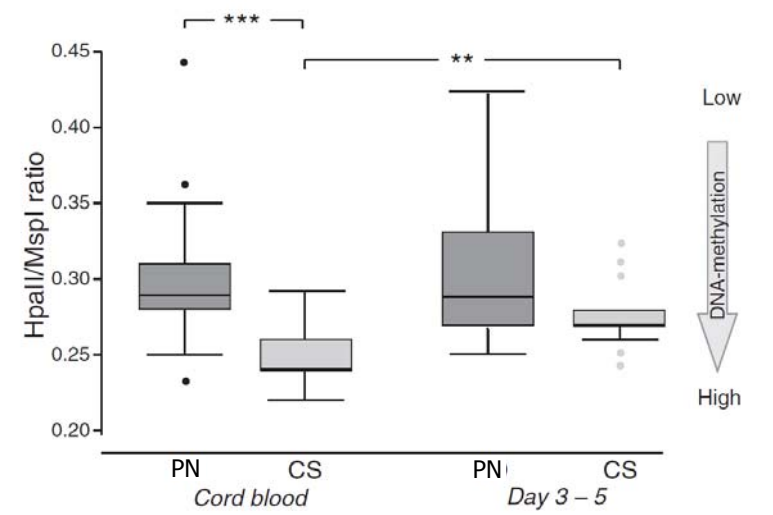
Decker E, Pediatrics 2010

REGULAR ARTICLE

Epigenetic modulation at birth – altered DNA-methylation in white blood cells after Caesarean section

T Schlinzig[1], S Johansson[2], A Gunnar[3], TJ Ekström[3], M Norman (mikael.norman@ki.se)[1]

1. Department of Clinical Science, Intervention and Technology, Karolinska Institute, Stockholm, Sweden
2. Department of Woman & Child Health, Karolinska Institute, Stockholm, Sweden
3. Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden



Karolinska Institutet

Wizard

MEDIA REPORT

JULY ISSUE 2009

ACTA PAEDIATRICA

C-section births cause genetic changes that may increase odds for developing diseases in later life

Press release issued 29 June 2009

This press release was issued in English and Swedish and both versions received MASSIVE coverage. This report focuses on the English coverage and we have attached a separate report, prepared by the AP editorial office which contains some of the Swedish coverage. Coverage included:

- Forbes Magazine (major US title)
- Business Week (major US title)
- Discovery Health (USA)
- Daily Telegraph (UK daily newspaper)
- Association of Reproductive Health Professionals
- Centers for Disease Control and Prevention (major US Government website)
- WomensHealth.gov (US Dept of Health)
- Medline Plus (US National Library of Medicine and National Institutes of Health)
- HealthFinder.gov (US Dept of Health)
- HealthDay News (provides news for more than 4,000 Internet and Intran Web sites)
- CBS network of TV websites (USA)
- NBC network of TV websites (USA)
- Fox News network of TV websites (USA)
- ABC network of TV websites (USA)
- Large number of regional TV websites (USA)
- World News Network (international syndicated news in 50 languages
- National Examiner (USA)
- Canada.com
- Montreal Gazette (Canada)
- Science Daily
- Caesarean Awareness Network (Australia)
- Sydney Midwife (Australia)
- Nursing Knowledge
- A Pure Genesis (parenting news)
- Absolute Life Moms
- All Refer Health
- Allergy Medicine (USA)
- American Academy of Anti-Aging Medicine
- Austin American-Statesman (USA)
- Baby Center (parenting news)
- Bester News (information portal)
- Bio Medicine
- Biocetera (life sciences resource)
- BioMedicine.org
- BioPortfolio News
- Birth Rhythm (childbirth blog)
- Calgary Herald (Canada)
- Creighton University Medical Center (USA)
- Cumhuriyet Portal (Turkey)
- Discovery Health (part of the Discovery channel
- Doctors Hangout (medical news and chat forum
- Drugs.com (information portal)
- Earth Times
- Economy Poslovni Portal (Serbia)
- Empowher.com (women's
- eScience News
- European Pressphoto Age
- Expert Answer (medical ne
- Facebook (social networkir
- Feedzilla.com (newsfeed)
- Haydon & Allen Valleys He
- Health on the Net Foundati
- Health Scout
- Insciences Organisation (m
- Karolinska Institutet (Swed
- Las Vegas Now
- Lenta.ru (news website, Ru
- MD Anderson Cancer Cent
- MedCastle.com (medical n
- Medical News Today
- Medicine Online
- MediLexicon
- Medscape Medical News
- Monsters & Critics (health
- My Online Wellness
- Nursing Birth (blog)
- Pluska.sk (medical news, S
- Pregnancy Guide
- Pregnancy Weekly
- Press Online (Serbia)
- Sakthi Foundation (natural healing news, USA)
- StaffNurse.com (nursing news, UK)
- SteadyHealth News
- Summit Medical Group (USA)
- Surfwax.com (newsfeed)
- The Star Phoenix
- Times Colonist (Canada)
- Times of India
- Twitter (social networking)
- US News & World Report
- WEB Beteg (medical news, Hungary)
- Web MD
- Webindia 123
- Wellsphere Health News
- Yahoo! News
- Your Health Connection
- Your Total Health (USA)

…from the Karolinska Institute, in Stockholm, said: "Delivery by **C-section** has been associated with **increased allergy, diabetes** and leukaemia risks

"Although the underlying cause is unknown, our theory is that **altered birth conditions** could cause a **genetic imprint** in the immune cells that could play a role later in life", reports *The Telegraph*

Epigenetic basis for Rheumatoid arthritis

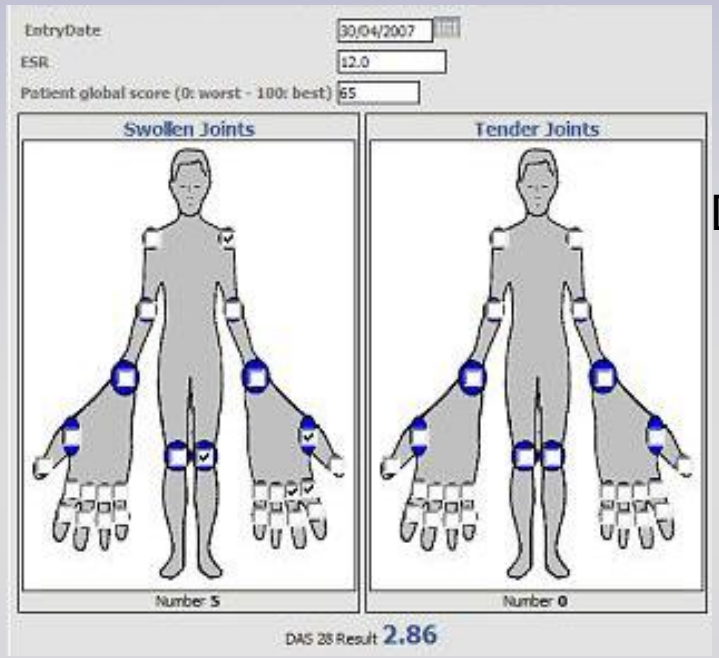
What is the genetic/epigenetic association with disease and phenotype?

- Over 2,000 new cases appear per year in Sweden,
- Prevalence about 0.7% of the population
- Lifetime risk to develop RA is over 2%.
- A correlation between RA, HLA type and smoking
- The direct and indirect anual costs for society have been estimated to 850 million € (Sweden 2001)

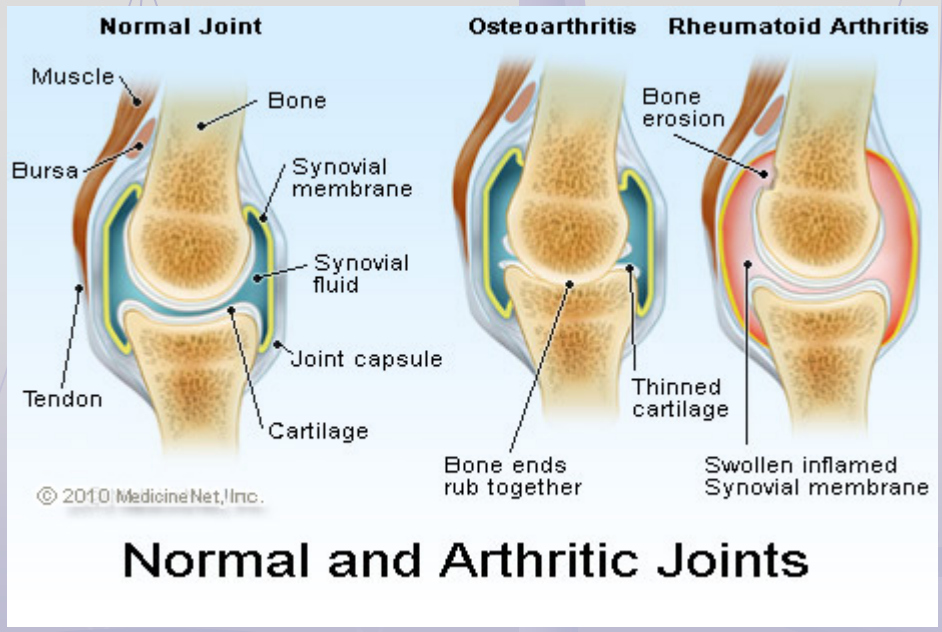
Q. Which associations exist between genome-wide epigenetic profiles, the genotype, and the life style/exposures (in particular smoking)?

Approach. Methylation analysis of large RA cohorts and discordant MZ twins in combination with exhaustive patient data

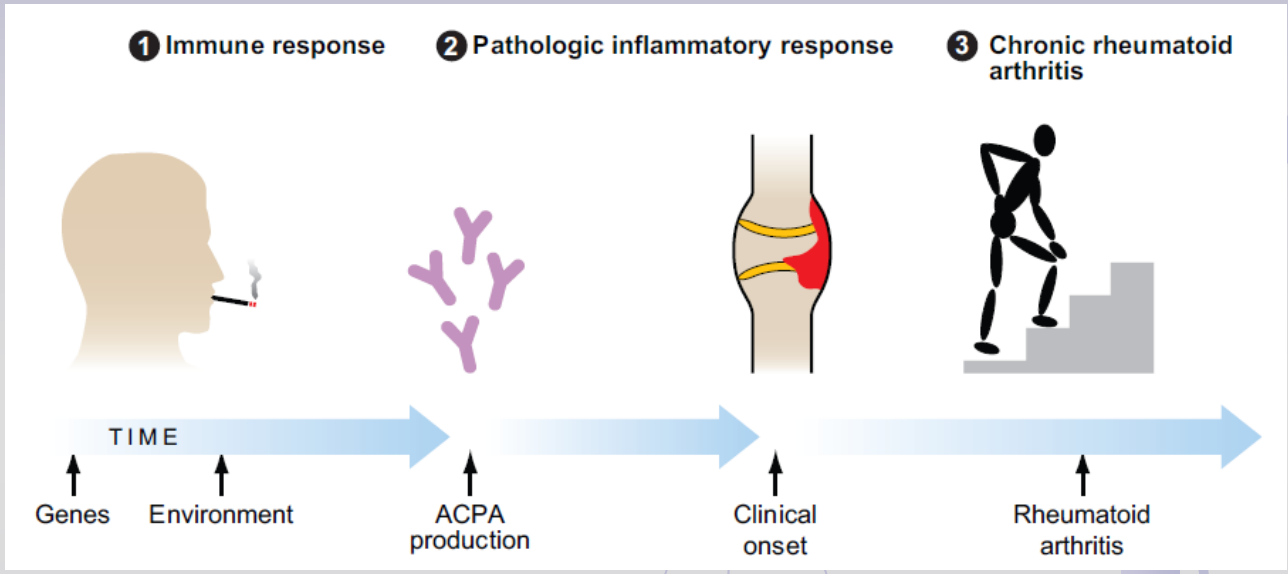
Rheumatoid arthritis; epigenetics as a novel paradigm for pathogenesis and phenotype

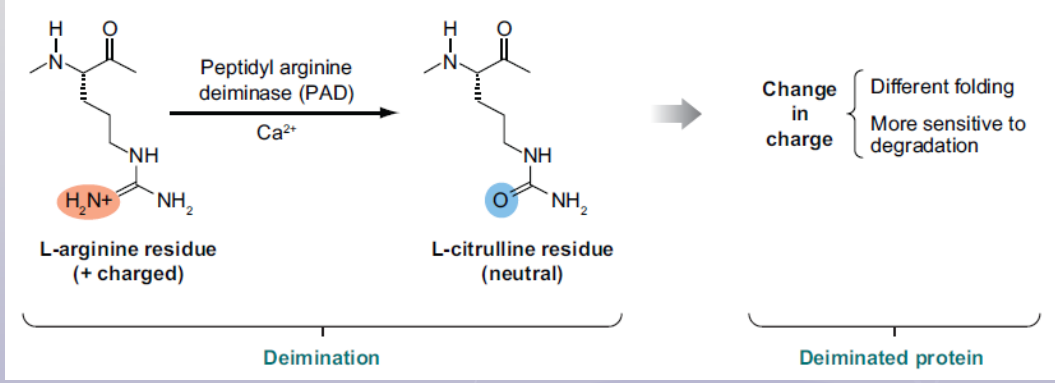


Disease Activity Score, DAS28



Environmental impact on development of ACPA and Rheumatoid arthritis





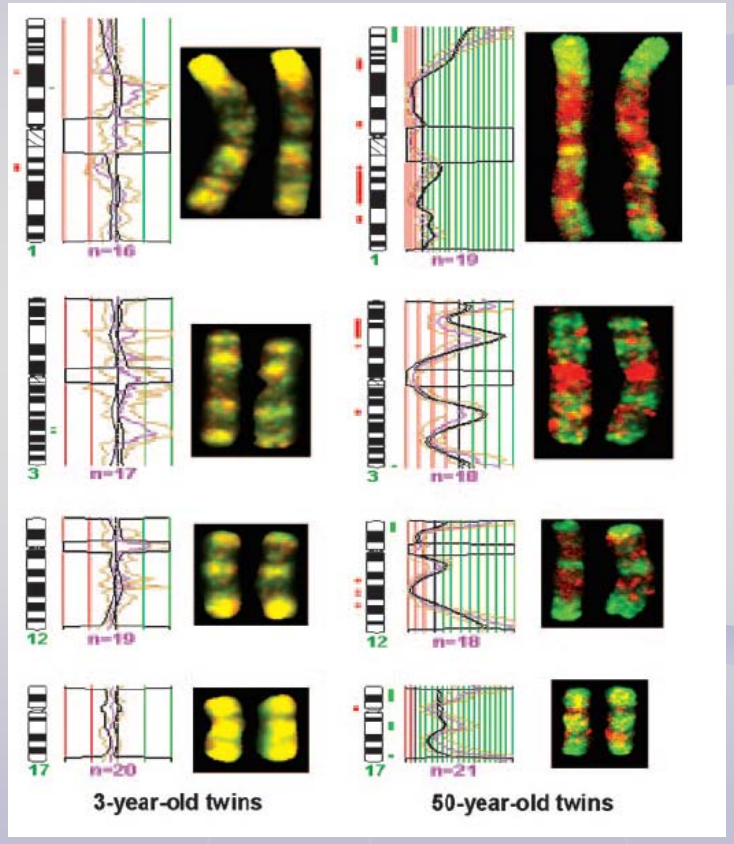
Citrullinated proteins exist normally in healthy individuals as well

Klareskog et al. Annu. Rev. Immunol. 2008. 26:651–75

The dynamics of epigenetics

Age dependent DNA-methylation divergence

in monozygotic twins



Karolinska Institutet

IMU Nov. 2011

Mario F. Fraga et al., PNAS 2005

Genetic predisposition to epigenetic change

Intra-individual Change Over Time in DNA Methylation With Familial Clustering

Hans T. Bjornsson, MD, PhD
Martin I. Sigurdsson, MD
M. Daniele Fallin, PhD
Rafael A. Irizarry, PhD
Thor Aspelund, PhD
Hengmi Cui, PhD
Wenqiang Yu, PhD
Michael A. Rongione, BA
Tomas J. Ekström, PhD
Tamara B. Harris, PhD
Lenore J. Launer, PhD
Gudny Eiriksdottir, PhD
Mark F. Leppert, MD
Carmen Sapienza, PhD
Vilmundur Gudnason, MD, PhD
Andrew P. Feinberg, MD, MPH

Context Changes over time in epigenetic marks, which are modifications of DNA such as by DNA methylation, may help explain the late onset of common human diseases. However, changes in methylation or other epigenetic marks over time in a given individual have not yet been investigated.

Objectives To determine whether there are longitudinal changes in global DNA methylation in individuals and to evaluate whether methylation maintenance demonstrates familial clustering.

Design, Setting, and Participants We measured global DNA methylation by luminometric methylation assay, a quantitative measurement of genome-wide DNA methylation, on DNA sampled at 2 visits on average 11 years apart in 111 individuals from an Icelandic cohort (1991 and 2002-2005) and on average 16 years apart in 126 individuals from a Utah sample (1982-1985 and 1997-2005).

Main Outcome Measure Global methylation changes over time.

Results Twenty-nine percent of Icelandic individuals showed greater than 10% methylation change over time ($P < .001$). The family-based Utah sample also showed intra-individual changes over time, and further demonstrated familial clustering of methylation change ($P = .003$). The family showing the greatest global methylation loss also demonstrated the greatest loss of gene-specific methylation by a separate methylation assay.

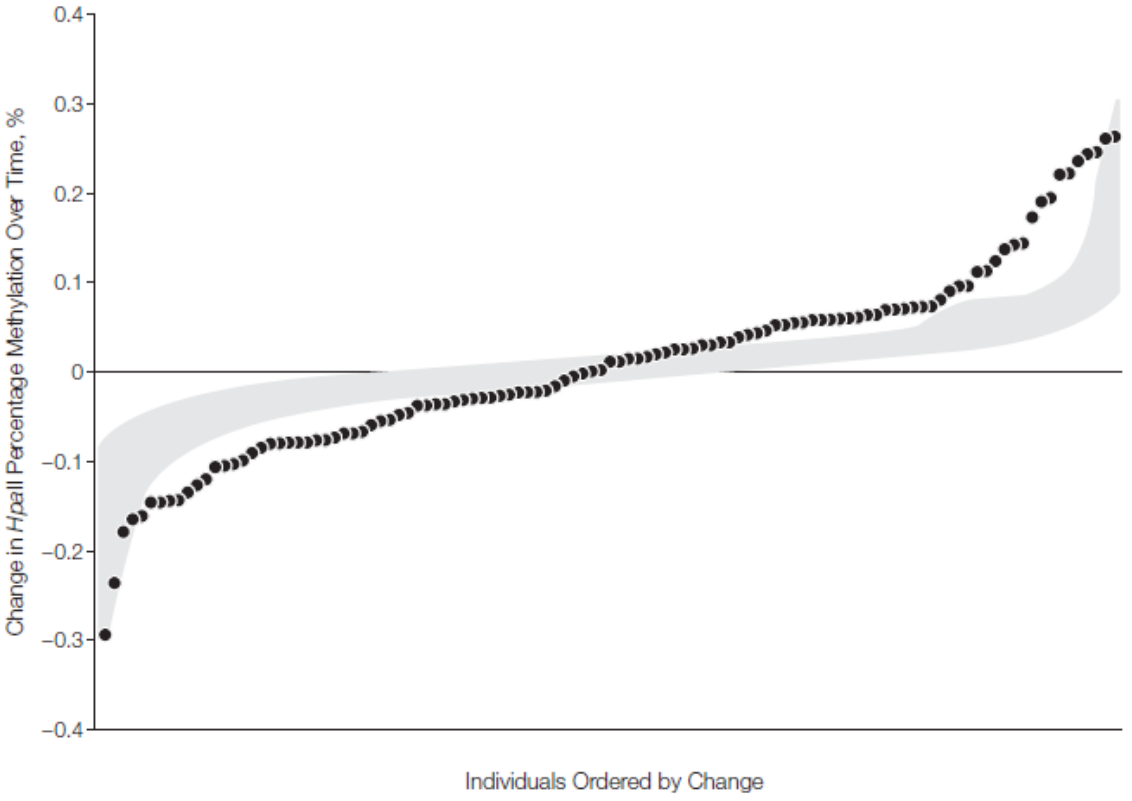
Conclusion These data indicate that methylation changes over time and suggest that methylation maintenance may be under genetic control.

EPIGENETIC MARKS ARE MODIFI-

JAMA. 2008;299(24):2877-2883 www.jama.com

Global DNA-methylation analysis of an Icelandic cohort

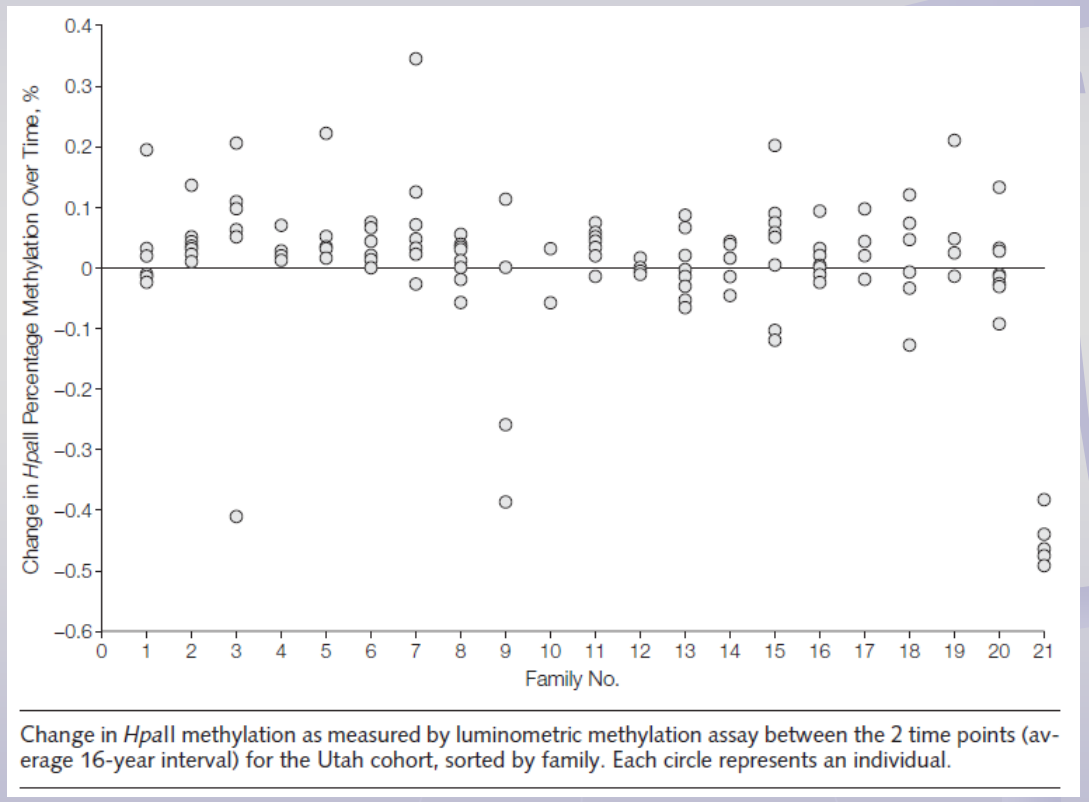
Figure 1. Global Change in *Hpa*II Methylation in Individuals Over Time in the Iceland Cohort



DNA methylation was measured by luminometric methylation assay between the 2 time points for the Iceland cohort (circles). Each circle represents an individual, and they are ranked on the x-axis based on the magnitude and direction of the change. The y-axis shows the absolute change between time points in the global percentage of methylation. The range of results from permutations of the time points within individuals, emulating the null hypothesis of no time effects, are shown as well (tinted region).

Bjornson et al. JAMA, 2008

LUMA analysis of a Utah cohort



Change in *Hpa*II methylation as measured by luminometric methylation assay between the 2 time points (average 16-year interval) for the Utah cohort, sorted by family. Each circle represents an individual.

Bjornson et al. JAMA, 2008

Illumina Golden Gate gene-specific analysis of a Utah family

Table 2. Genes Showing Greatest Differences in Family 21 and Across All Individuals, Showing a High Representation of Immunological Mediators Among These Genes

Gene Symbol	Gene Name	Gene Function
AIM2[a]	Absent in melanoma 2	Interferon-γ–inducible transcript
CSF3R[a]	Colony-stimulating factor 3 receptor	Colony-stimulating factor 3 receptor
HOXA5	Homeobox A5	Hox gene
PTK6	Protein tyrosine kinase 6	Protein, tyrosine kinase
ERCC3	Excision repair cross-complementing rodent 3	Helicase with excision repair functions
LMO2[a]	LIM domain only 2	Role in erythropoiesis and in T-cell leukemogenesis
SYK[a]	Spleen tyrosine kinase	Spleen, tyrosine kinase
IL10[a]	Interleukin 10	Cytokine
BIRC4	Baculoviral IAP repeat–containing protein 4	Apoptosis inhibitor
IL16[a]	Interleukin 16	Cytokine
LCN2[a]	Lipocalin 2	Protein associated with neutrophil gelatinase
TRIP6	Thyroid receptor–interacting protein 6	Regulates lysophosphatidic acid–induced cell migration
EMR3[a]	egf-Like module–containing mucin-like receptor 3	Myeloid-myeloid interactions during immune and inflammatory responses

[a] Genes that play a role as immunological modulators.

Bjornson et al. JAMA, 2008

Is there any association of the GENETIC information with the EPIGENETIC, which can influence RA disease RISK?

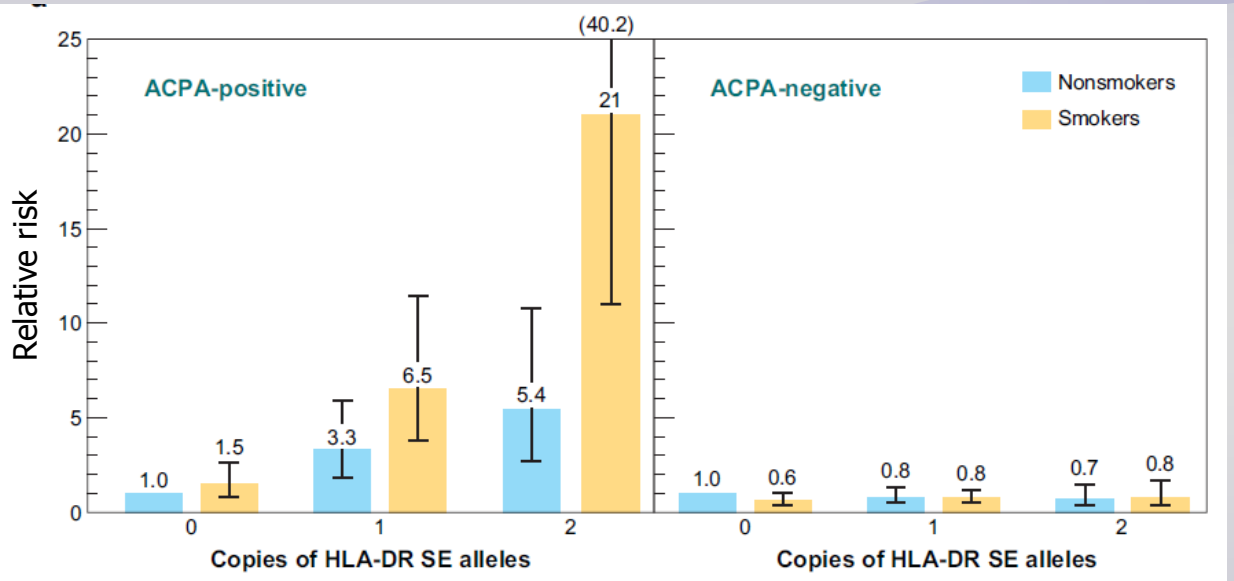
Is there any association of the GENETIC information with the EPIGENETIC, which can influence RA disease PHENOTYPE?

Identification of differentially methylated regions (DMR) in MZ twins discordant for rheumatoid arthritis

CHARM algorithm modified for paired analysis

IMU Nov. 2011

Environmental (smoking) impact on development of ACPA and Rheumatoid arthritis in a large Swedish cohort



Current analysis: Can smoking explain methylation changes and disease risk in the RA discordant MZ twin cohort?

Klareskog et al. Annu. Rev. Immunol. 2008. 26:651–75

Is there any association of the GENETIC information with the EPIGENETIC, which can influence RA disease RISK?

Is there any association of the GENETIC information with the EPIGENETIC, which can influence RA disease PHENOTYPE?

Acknowledgements

Mohsen Karimi

Malin Almgren

Louise Sjöholm

Andy Feinberg

Martin Aryee

Yun Liu

Cecilia Söderberg-Nauclér

Klas Stråål

Afsar Rahbar (Maral)

Jesper Tegnér

David Gomez-Cabrero

Vladana Vukojević

Mikael Norman

Titus Schlintzig

Lars Klareskog

Leonid Padykov

Aase Hensvold

Anca Catrina

Karolinska Institutet