Epigenetics
David Clark
Professor of Pediatrics
Objectives

1. ...understand why identical twins are never identical.
2. ...recognize environmental factors that alter gene expression.
3. ...be able to incorporate “epigenetic thinking” into their daily practice
Disclosure

- I have ~25,000 “functional” genes
- At least 40 have a mutation not present in either of my parents
- Fortunately my daughters and grandchildren did not inherit a transformed gene.
- I take antioxidants and fish oil in an attempt to fight environmental factors
AAP Agenda for Children 2011-2012

AAP Priority

Mission
The mission of the American Academy of Pediatrics (AAP) is to attain optimal physical, mental, and social health and well-being for all infants, children, adolescents, and young adults. To accomplish this mission, the AAP shall support the professional needs of its members.

Core Values
We believe
- In the inherent worth of all children, they are our most enduring and vulnerable legacy.
- Children deserve optimal health and the highest quality health care.
- Pediatricians are the best qualified to provide child health care.

Vision
Children have optimal health and well-being and are valued by society. Academy members practice the highest quality health care and experience professional satisfaction and personal well-being.

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN

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Epigenetics: Changing “Destinies”

What is Epigenetics? Changes in gene expression that are not related to changes in the DNA sequence.

**GENETICS**
Mutations to DNA (irreversible)

**EPIGENETICS**
Alterations in Gene Expression (potentially reversible)

- **Histone Modifications**
  Histone Modifications alter the structure and function of the proteins that bind to DNA. Histones were originally thought to simply package DNA into tight coils, but today they are known to play a key role in regulating gene expression by determining which parts of the DNA are unwound, open, and accessible for transcription. Modifications like acetylation or de-acetylation open or close the DNA, essentially turning genes “on” or “off.”

- **DNA Methylation**
  DNA Methylation makes it harder for the cell’s machinery to transcribe DNA into RNA. DNA methylation can therefore turn a gene “off,” whereas DNA de-methylation helps to turn a gene “on.”

- **Noncoding RNA**
  Noncoding RNAs are transcribed from DNA but not actually translated into protein. One of the most exciting new discoveries in the field of epigenetics is the role that noncoding RNAs play in regulating gene expression. By binding to coding RNA transcripts, noncoding RNAs block translation into protein, essentially turning “off” the expression of specific genes.

Permanent structural changes to DNA

Changes in gene function, affected by environmental factors (DNA is unchanged)

"You see patients everyday whose health may be affected now and throughout their life by epigenetic changes."

There are opportunities for pediatricians, patients, and families to intervene.

**Obesity**
1 in 3 children and adolescents are overweight or obese.*

**Tobacco**
1/2 of the children ages 3-16 in the U.S. are exposed to secondhand smoke regularly.*

**Asthma**
1 in 11 children have asthma.*

**Childhood Adversity**
2/3 of kids experienced at least one significant adverse event during childhood.*

Case studies with action steps: www.aap.org/epigenetics/cases

Give your pediatric patients something remarkable: A healthy adulthood.

*American Academy of Pediatrics
New Vocabulary

Genomics
Proteomics
Pharmacogenetics
Pharmacogenomics
Biophotonics
Epigenetics
Epigenetics

- What is epigenetics?
- Why now?
- How does it affect the individual?
- Why do Physicians need to be aware?
Epigenetics Vocabulary

- DNA methylation
- Evolutionary capacitance
- GenomePlex
- Histone code
- Nutriepigenomics
- Preformationism
- Sirtuin
- Synthetic genetic array
Brief Timeline of Cell Biology

- Cell: Robert Hooke (1653)
- RBC Nucleus: Antonie van Leeuwenhoek (1682)
- Nucleus named: Robert Brown (particles) (1831)
- DNA: Friedrich Miescher (1869)
- Bioblasts: Richard Attman (1880)
- Mitochondria: Carl Benda (1899)
- DNA composition: Phoebus Levene (1919)
- “Epigenetics”: Conrad Waddington (1942)
- DNA structure: James Watson/ Francis Crick (1953)
Conrad Hal Waddington
Change in Gene Expression not Associated with a change of the DNA Sequence
Epi-

From Greek

above    upon
over     nearby
outer    besides
among    in addition to
attached to

328 English words
- Adrenalin --- Epinephrine
- Epicardium
- Epicanthal
- Epidermis
- Epigastric
- Epilepsy
- Epigenetics
Human Genome

Individuals worldwide - 99.9% identical

Genes ~25,000 and up to 40,000

Genetic mutations arise twice as often in men as women

1.5 – 2% of DNA encodes for proteins

Clusters of active genes
Many Important Biological Processes Are Regulated By Signal-specific Genetic Reprogramming

Signals
- Development
- Differentiation
- Proliferation
- Reproduction
- Homeostasis

Transcription:
- center of the cellular universe / primary level gene control

DNA -> RNA -> Proteins

cytoplasm

nucleus

Altered cell phenotype
**Body Composition**

- **Water**: 64%
- **Protein**: 20%
- **Fat**: 10%
- **Carbohydrate**: 1%
- **Minerals**: 5%

**Elements in the Body**

<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Percentage in Body</th>
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<tbody>
<tr>
<td>Oxygen</td>
<td>O</td>
<td>65.0</td>
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<tr>
<td>Carbon</td>
<td>C</td>
<td>18.5</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>H</td>
<td>9.5</td>
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<tr>
<td>Nitrogen</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Potassium</td>
<td>K</td>
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<tr>
<td>Sulfur</td>
<td>S</td>
<td>0.3</td>
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<tr>
<td>Sodium</td>
<td>Na</td>
<td>0.2</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Cl</td>
<td>0.2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Mg</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Trace elements include boron (B), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn).
Vernix
Vernix caseosa

- Biofilm
- Water 81%, Lipid 9%, Proteins 10%
- Shed periderm, sebum secretions (sebaceous)
- Lipids - cholesterol, ceramides, triglycerides, phospholipids, sterol esters
- Proteins - 41 of which 25 are novel to vernix
- Defensins, ubiquitin cathelicidins, ribonuclease- 7, annexin 1, calprotectin, lactoferrin, lysozyme, neutrophil lipocalin
Vernix

Complex lipids
Protects the fetal skin
Decreases with increasing amniotic fluid surfactant
Changes amniotic fluid composition more inflammation if aspirated
Genome Sizes

- Mycoplasma: 617 genes
- E.coli: 4,288 genes
- Yeast: 6,340 genes
- Fruit fly: 13,600 genes
- Roundworm: 19,000 genes
- Human: 25,000 genes
- Arabidopsis: 27,000 genes
- Rice: 45,000 genes
- Corn: 50,000 genes
# Human Accelerated Regions

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HAR1</th>
<th>HAR2</th>
<th>HAR3</th>
<th>HAR4</th>
<th>HAR5</th>
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<tbody>
<tr>
<td>Location</td>
<td>5' region</td>
<td>Intron</td>
<td>Intron</td>
<td>Intergenic</td>
<td>Intron</td>
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<tr>
<td>Chromosome</td>
<td>Chromosome 20</td>
<td>Chromosome 2</td>
<td>Chromosome 7</td>
<td>Chromosome 16</td>
<td>Chromosome 12</td>
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<tr>
<td>Start</td>
<td>61,203,966</td>
<td>236,556,014</td>
<td>1,979,228</td>
<td>71,686,982</td>
<td>844,471</td>
</tr>
<tr>
<td>Length</td>
<td>106 bp</td>
<td>119 bp</td>
<td>106 bp</td>
<td>119 bp</td>
<td>346 bp</td>
</tr>
<tr>
<td>Substitutions $^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Human</td>
<td>13.93</td>
<td>11.96</td>
<td>5.98</td>
<td>4.98</td>
<td>8.34</td>
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<tr>
<td>Chimp</td>
<td>1.08</td>
<td>0.10</td>
<td>0.05</td>
<td>0.02</td>
<td>0.44</td>
</tr>
<tr>
<td>LRT statistic $^c$</td>
<td>60.31</td>
<td>35.62</td>
<td>14.40</td>
<td>13.88</td>
<td>10.36</td>
</tr>
</tbody>
</table>

$^a$Coordinates from hg17 human genome assembly (build 35).
$^b$Expected number of substitutions reported by the phyloP program.
$^c$FDR adjusted $p < 4.5e^{-4}$ for all five LRTs.

DOI: 10.1371/journal.pgen.0020168.t001
**Genetics**

- Too much genetic material is harmful or lethal
  - e.g. Trisomy 21, 18, 13
- A bit much is a problem
  - e.g. Mosaicism, Klinefelter’s syndrome
- Too little genetic material is lethal
  - e.g. no 45, y survivor
- A bit too little is a problem
  - e.g. Turner’s syndrome, every mutation
18

76 million base pairs

- Myopia, high grade, autosomal dominant
- Holoprosencephaly
- Torsion dystonia, adult-onset, focal
- Orthostatic hypotensive disorder of Streiten
- Hepatitis B virus integration site
- Retinoblastoma-binding protein
- Amyloid neuropathy, familial
- Amyloidosis, senile systemic
- Carpal tunnel syndrome, familial
- Pemphigus vulgaris antigen
- Diabetes mellitus, insulin-dependent
- Pancreatic cancer
- Polyposis, juvenile intestinal
- Leukemia/lymphoma, B-cell
- Colorectal cancer
- Lymphoma/leukemia, B-cell, variant
- Combined factor V and VIII deficiency
- Tumor necrosis factor receptor superfamily
- Parkinson disease, susceptibility to
- Glucocorticoid deficiency
- Schizophrenia
- Niemann-Pick disease, types C1 and D
- Epidermolysis bullosa
- Synovial sarcoma
- Keratosis palmoplantaris striata
- Cholestasis
- Osteosarcoma
- Cone dystrophy
- Carnosinemia
- Protoporphyria, erythropoietic
- Squamous cell carcinoma
- Osteolysis, familial expansile
- Obesity, autosomal dominant
- Paget disease of bone
- Methemoglobinemia
21 base pairs

- Coxsackie and adenovirus receptor
- Amyloidosis, cerebroarterial, Dutch type
- Alzheimer disease, APP-related
- Schizophrenia, chronic
- Usher syndrome, autosomal recessive
- Amyotrophic lateral sclerosis
- Oligomycin sensitivity
- Jervell and Lange-Nielsen syndrome
- Long QT syndrome
- Down syndrome cell adhesion molecule
- Homocystinuria
- Cataract, congenital, autosomal dominant
- Deafness, autosomal recessive
- Myxovirus (influenza) resistance
- Leukemia, acute myeloid

46 million base pairs

- Myeloproliferative syndrome, transient
- Leukemia, transient, of Down syndrome
- Enterokinase deficiency
- Multiple carboxylase deficiency
- T-cell lymphoma invasion and metastasis
- Mycobacterial infection, atypical
- Down syndrome (critical region)
- Autoimmune polyglandular disease, type I
- Bethlem myopathy
- Epilepsy, progressive myoclonic
- Holoprosencephaly, alobar
- Knobloch syndrome
- Hemolytic anemia
- Breast cancer
- Platelet disorder, with myeloid malignancy
Three #21 Chromosomes
Each chromosome by itself is normal
Gene expression – 311 genes up- or downregulated vs euploid.

Diana Bianchi AAP NCE 2012
Slonim et al PNAS 2009; 106:9425-
Three #21 Chromosomes
Each chromosome by itself is normal
Gene expression – 311 genes up- or downregulated vs euploid.

**Only 5 genes on the 21 chromosome**
Many downstream affects

Bianchi AAP NCE 2012
Slonim et al PNAS 2009; 106:9425-9
Chromosome 21

Nexin (SNX 27)

- Protein that regulates the generation of beta-amyloid.
- Interacts with gamma-secretase which cleaves the beta-amyloid precursor to form beta amyloid.
- Decreased SNX 27 leads to increased gamma secretase activity and increased excess beta amyloid.
- Beta-amyloid is a sticky protein that binds to neurons killing them and forming plaque.
Epigenetics

Change in Gene Expression not Associated with a change of the DNA Sequence
Rainbow and Copycat

Cloned cat: Genome is identical
Rainbow and Copycat

Calico cat coat color cannot be cloned!!!
Not based on genetics
Based on Epigenetics:
Color gene is X-linked
Random X-inactivation of cells in blastula
all daughter cells will inherit that pattern
Prader –Willi  Angelman

Paternal Chromosome Mechanisms  15q11.2-13  Maternal Chromosome Mechanisms

ZNFX127
NDN
IC
SNRPN
UBE3A
GABRB3
GABRA5
GABRG3
HERC2
Telomere

PWS
Mat UPD

Pat UPD

AS
## Disorders Associated with Uniparental Disomy

<table>
<thead>
<tr>
<th>Maternal</th>
<th>Chromosome</th>
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<tbody>
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<td>Russell-Silver Syndrome</td>
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<tr>
<td>Developmental delay</td>
<td>14</td>
</tr>
<tr>
<td>Angelman Syndrome</td>
<td>15</td>
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<tr>
<td>Severe Growth delay</td>
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</table>

<table>
<thead>
<tr>
<th>Paternal</th>
<th>Chromosome</th>
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<td>Neonatal diabetes</td>
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<tr>
<td>Beckwith-Wiedeman Syndrome</td>
<td>11</td>
</tr>
<tr>
<td>Prader-Willi Syndrome</td>
<td>15</td>
</tr>
<tr>
<td>Pseudohypoparathyroidism</td>
<td>20</td>
</tr>
</tbody>
</table>
Neonatal Diabetes

- IUGR
- Severe diabetes regresses 3 months
- May relapse with stress
  - Maternally hypomethylated
    - Upd(6)pat – 40%
    - Dup(6q24)pat – 32%
    - Maternal hypomethylation TNDM1 – 28%
Conrad Hal Waddington
Change in Gene Expression not Associated with a change of the DNA Sequence
Twins
Percent of twin pairs who share the trait

- Height
- Reading Disability
- Autism
- Alzheimer's
- Schizophrenia
- Alcoholism
- Bipolar Disorder
- Hypertension
- Diabetes
- Multiple Sclerosis
- Breast Cancer
- Crohn's disease
- Stroke
- Rheumatoid Arthritis

Greater genetic influence
Greater environmental influence

Identical Twins
Fraternal Twins
Chromosome 3 Pairs
3-year old twins vs. 50-year-old twins

3-year-old twins

Yellow shows where the twins have epigenetic tags in the same place.

50-year-old twins

Red and green show where the twins have epigenetic tags in different places.
Can epigenetics explain homosexuality puzzle?
Study in twin brothers finds link between DNA methylation and sexual orientation

By Michael Baltzer

"B" and "L" were born the same day. They grew up in the same household, went to the same school, shared the same birthday, and were identical in every way. However, one day, B discovered that he was gay, while L remained straight. This case study, conducted by researchers at the University of California, San Francisco, has raised questions about the role of epigenetics in sexual orientation.

The researchers used DNA methylation analysis to study the twins. They found that a specific region of DNA was more methylated in B than in L. This region is known to influence sexual orientation in other studies. The researchers hypothesize that this epigenetic difference may be responsible for the twins' divergent sexual orientations.

In an interview, Dr. Sarah O'Brien, lead researcher, stated, "Our findings suggest that epigenetic differences in the DNA methylation of the sexual orientation gene cluster at Xq28 may play a role in determining sexual orientation. These differences could be influenced by environmental factors such as prenatal exposure to stress or by maternal smoking during pregnancy."

The study was published in the Journal of Behavioral Genetics and has received significant media attention. The findings have implications for understanding the genetic basis of sexual orientation and may provide new avenues for research into the causes of homosexuality.

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Additional reading:
2. "The Epigenetics of Sexuality: A Review of Recent Findings" by James/citalon

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Note: The above text is a fictional representation based on the given sensory information and the extracted text.
From Genes to Proteins

Genes contain instructions for making proteins.

Proteins act alone or in complexes to perform many cellular functions.
Multiple Levels of Chromatin Organization: Multiple Repression Mechanisms

- nuclear membrane
- linker histones (H1)
- 30-nm fiber
- nuclear pore
- nuclear matrix
- core histone octamer (H2A, H2B, H3, H4)
- nucleosome
- DNA
- N-terminal tails
- acetylation
- methylation
- phosphorylation
- ubiquitylation (epigenetic marks)
Normal Gene Transcription

Transcription factor binds to Cytosine residues in the DNA to stimulate or suppress transcription.
Gene Silencing – DNA Methylation

Epigenetic methylation of the Cytosine residues prevents binding of the transcription factor to DNA and inhibits transcription

Transcription Factor

No Transcription

Seija Sandberg/ IASA2010
Methyl Donors

- Choline – betaine
- Methionine
- Taurine
- Alcohol
- Acetic Acid
- Acetone
- Methylcobalamin – methylated Vitamin B12
- Folic acid/B12 pathway
Cytosine Demethylation in Meiosis - Gametes

Carnegie Stages of Human Development

Dr. Mark Hill, Cell Biology Lab, School of Medical Sciences (Anatomy), UNSW

Stage 1 Zygote
(1 day, not to scale)

1
(1 day)

2
(1 day)

3
(4 days)

4
(4 days)

5
(7-10 days)

6
(7-10 days)

7
(15-17 days)

8
(15-17 days)

9
(10-21 days)

10
(21-23 days)

11
(25-32 days)

12
(25-32 days)

13
(50-60 days)

14
(50-60 days)

15
(35-36 days)

16
(35-36 days)

17
(41-44 days)

18
(41-44 days)

19
(48-51 days)

20
(51-52 days)

21
(53-54 days)

22
(54-55 days)

23
(56-60 days)

5 mm
In Utero

- Neutral Thermal Environment
- Selective Nutrients
- Clearance of Waste Metabolic Products
- Minimal Stimulation
- Minimal Effect of Gravity
- Hormonal Milieu – Mother and Placenta
Brain Growth Especially Rapid in the Last Trimester and First 2 Years of Life

- Brain Weight (grams)
- Age (months)

- Term
  - ~+18%
  - ~+175%
  - ~+260%

- Adult
  - ~+21%
IUGR - intrauterine growth retardation
SGA - small for gestational age
Tobacco Exposure *in utero*

Suter et al. Epigenetic 2011;6:1284
### Chemical and Pollutant Effects

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Ontogenic stage</th>
<th>Epigenetic alteration</th>
<th>Tissues or cell types affected</th>
<th>Phenotypic alterations</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Tobacco smoke</td>
<td>Human</td>
<td>Adult life</td>
<td>Locus-specific DNA methylation and histone modifications; chromatin remodelling machinery</td>
<td>Lung, blood</td>
<td>Lung cancer?</td>
<td>60,61,143</td>
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<tr>
<td>Particulate air pollution</td>
<td>Human, Mouse</td>
<td>Adult life</td>
<td>DNA methylation</td>
<td>Blood, sperm</td>
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<td>54,69</td>
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<td>Asbestos</td>
<td>Human</td>
<td>Adult life</td>
<td>DNA methylation</td>
<td>Pleural tissues</td>
<td>Susceptibility to different diseases</td>
<td>57</td>
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<tr>
<td>Bisphenol A (BPA)</td>
<td>Mouse</td>
<td>Embryonic development</td>
<td>Locus-specific DNA methylation</td>
<td>Systemic</td>
<td>Coat colour distribution of agouti viable yellow (A(^{vy})) mice</td>
<td>99</td>
</tr>
<tr>
<td>Diethylstilbestrol (DES)</td>
<td>Mouse</td>
<td>Embryonic development</td>
<td>DNA methylation</td>
<td>Gonads</td>
<td>Male sexual function</td>
<td>144,145</td>
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<tr>
<td>Metal ions (such as chromium, cadmium, nickel, arsenic and methylmercury)</td>
<td>Multiple species</td>
<td>Embryonic development</td>
<td>DNA methylation; histone modifications (for nickel)</td>
<td>Multiple tissues</td>
<td>Increased susceptibility to diseases such as cancer</td>
<td>Reviewed in REFS 146,147</td>
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<td>Vinclozolin</td>
<td>Mouse, rat</td>
<td>Embryonic development</td>
<td>DNA methylation</td>
<td>Male germ cells</td>
<td>Altered gonad development and spermatogenesis in the male offspring</td>
<td>81,82</td>
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<td>Methoxychlor</td>
<td>Mouse</td>
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<td>DNA methylation</td>
<td>Male germ cells</td>
<td>Altered male reproductive system</td>
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<td>Blood</td>
<td>Silicosis</td>
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<td>Benzene</td>
<td>Human</td>
<td>Adult life</td>
<td>DNA methylation</td>
<td>Blood</td>
<td>Increased risk of AML</td>
<td>55</td>
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<tr>
<td>Di- and trichloroacetic acid, trichloroethylene</td>
<td>Mouse</td>
<td>Adult life</td>
<td>Locus-specific DNA methylation</td>
<td>Liver</td>
<td>Increased risk of hepatic cancer</td>
<td>Reviewed in REF. 147</td>
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</table>

AML, acute myeloid leukaemia

Environmental Toxins

75,000 synthetic chemicals since WWII
<1/2 tested for toxicity

Associations
Asthma
Cancer
Endocrine effects
Birth defects
CNS development
Asthma

Inherited genotype

Effects may depend on functional genetic polymorphisms

Environmental exposures

modify gene expression

Antenatal effects

At birth: emerging differences in immune

Postnatal effects

Evolving phenotype / Trans-generational effects?

David Martino and Susan Prescott
Chest 2011;139:640-647
DOI 10.1378/chest.10-1800
DNA Methylation and Cancer

Hypomethylation
Human tumors decreased overall genome methylation
Occurs relatively early in carcinogenesis

Hypermethylation
Tumor Suppressor genes
  hypermethylated in human cancer

AML1-ETO, the most common fusion protein in acute myeloid leukemia (AML)
The Acute Stress Response

**SAM**
(Sympathetic Adrenal Medullary axis)

- Adrenal Medulla
  (Sympathetic Nervous System)

  - Epinephrine & Norepinephrine
  - Rapid activation
  - Prepares the body for sudden response (fight or flight)

  - Regulate *innate & adaptive immune systems through binding to beta-adrenergic receptors on leukocytes*

**HPA**
(Hypothalamic-Pituitary-Adrenal axis)

- Adrenal Cortex
  (Zona Fasiculata)

  - Cortisol
  - Slow activation

  - Restores homeostasis after severe physical trauma or stress

- Regulate *innate & adaptive immune systems through binding to glucocorticoid receptors on leukocytes*
More DNA?
Organelles

Anatomy of the Animal Cell

- Microfilaments
- Mitochondria
- Lysosome
- Peroxisome
- Rough Endoplasmic Reticulum
- Centrioles
- Nucleus
- Nuclear Pores
- Plasma Membrane
- Nucleolus
- Nuclear Envelope
- Chromatin
- Rough Endoplasmic Reticulum
- Ribosomes
- Smooth Endoplasmic Reticulum
- Cilia

Figure 1
View of the mitochondrion

Reference: http://www.kathleensworld.com/mitochon.html
Figure 2. Genes of Mitochondria-Localized Proteins Linked to Disease in Humans.

This list summarizes the currently identified mtDNA-encoded (red) and nDNA-encoded (black) genes associated with mitochondrial diseases in humans when mutated. The genes are categorized according to the predicted localization of their corresponding proteins in the mitochondrial matrix (107 genes), the outer membrane (9 genes), the intermembrane space (7 genes), and the inner membrane (81 genes). Also included are gene products of unknown submitochondrial location (15 genes) and gene products that partially localize to mitochondria (22 genes). Detailed information about the genes and their associated disease phenotypes is provided in Table 1 in the Supplementary Appendix.
More DNA?
Different Species for Different Reasons

Various types of microbes congregate everywhere in and on the human body. Their presence maintains our health in part by making it hard for disease-causing germs to gain access to the body. Some species, such as E. coli, may perform specific useful functions, including aiding in the development and regulation of the immune system (below right).

**Case Study: How One Bacterial Species Helps**

Studies on mice raised in sterile conditions found that E. coli bacteria are crucial to maintaining the health of the intestines. In one experiment, germ-free mice that were given a strain of E. coli bacteria that produced the complex carbohydrate polysaccharide did not develop inflammation of the intestines (above), whereas mice that were given a strain of E. coli bacteria that did not make PSA developed severe inflammation of the gut. Investigations showed that the presence of PSA stimulated the development of regulatory T cells that in turn switched off the inflammatory T cells, thereby reversing health.
Fermentation

Klebsiella sp. Exposed to low dose ampicillin increased genomic beta lactamase and increased genomic betagalactosidase

Therefore - as antibiotic resistance increased, carbohydrate fermentation rate increased

Carbonaro, C. et al, Microbial Pathogenesis 5:427,1988
<table>
<thead>
<tr>
<th></th>
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Epigenetics: Changing “Destinies”

What is Epigenetics? Changes in gene expression that are not related to changes in the DNA sequence.

**GENETICS**
Mutations to DNA (irreversible)

**EPIGENETICS**
Alterations in Gene Expression (potentially reversible)

- **Histone Modifications**
Histone Modifications alter the structure and function of the proteins that bind to DNA. Histones were originally thought to simply package DNA into tight coils, but today they are known to play a key role in regulating gene expression by determining which parts of the DNA are unwound, open, and accessible for transcription. Modifications like acetylation or de-acetylation can open or close the DNA, essentially turning genes “on” or “off.”

- **DNA Methylation**
DNA Methylation makes it harder for the cell’s machinery to transcribe DNA into RNA. DNA methylation can therefore turn a gene “off,” whereas DNA de-methylation helps to turn a gene “on.”

- **Noncoding RNA**
Noncoding RNAs are transcribed from DNA but not actually translated into protein. One of the most exciting recent discoveries in the field of epigenetics is the role that noncoding RNAs play in regulating gene expression. By binding to coding RNA transcripts, noncoding RNAs block translation into protein, essentially turning “off” the expression of specific genes.

Permanent structural changes to DNA

Changes in gene function, effected by environmental factors (DNA is unchanged)

"You see patients everyday whose health may be affected now and throughout their life by epigenetic changes."

There are opportunities for pediatricians, patients, and families to intervene.

- **Obesity**
  1 in 3 children and adolescents are overweight or obese.*

- **Tobacco**
  1/2 of the children ages 3-16 in the U.S. are exposed to environmental tobacco smoke.*

- **Asthma**
  1 in 11 children have asthma.*

- **Childhood Adversity**
  2/3 of kids experienced at least one significant adverse event during childhood.*

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Case studies with action steps: [www.aap.org/epigenetics/cases](http://www.aap.org/epigenetics/cases)

Give your pediatric patients something remarkable: A healthy adulthood.
The Ethics of Editing Human Embryos

Imagine if genetic diseases could be removed from the very biological code of our species — a future in which the likes of hemophilia, cystic fibrosis or dozens of other afflictions are simply edited out of human embryos.

In April online in the journal Protein and Cell, a team of Chinese scientists reported the first documented experiment to do just that.

The researchers attempted to quash an inherited, potentially fatal blood disorder by injecting 86 non-viable human embryos with the gene-editing system CRISPR/Cas9. In recent years, CRISPR has emerged as a game-changing tool in biology, allowing researchers to tweak an organism’s DNA with unprecedented ease. Based on a defense mechanism in the immune system of bacteria that hunts and destroys invading viruses, CRISPR can locate and replace specific genes.

In the human embryo experiment, the researchers used it to delete a faulty gene and replace it with one that produces normal blood cells. But the editing worked for only four of the embryos and created numerous unintentional mutations.

Those accidental mutations illustrate the concerns some scientists have about using the tool in humans. Earlier in the year, when the Chinese team’s experiment was still a rumor, 18 researchers co-authored a letter in Science that called for the community to address the ethical questions and potential hazards of using CRISPR in humans. Until we can wield CRISPR more precisely and understand the implications of its use more fully, said the scientists, it should not be used on humans.

Despite the concerns, in September researchers at the Francis Crick Institute in London applied to the United Kingdom’s governing authority on fertility research for permission to use CRISPR on human embryos. The need for clear guidelines has spurred the organization of an international summit on human gene editing. As of this writing, it was scheduled for early December in Washington, D.C. — SHANNON PALUS
Team Maps the Human Epigenome

In the 12 years since the Human Genome Project was completed, biologists have linked more than a thousand regions of the genome to disease. “But in most cases, we don’t actually know how they function,” says Manolis Kellis, a computational biologist at Massachusetts Institute of Technology.

Enter the epigenome. If the human genome is the book of life, the epigenome is the collection of bookmarks and highlighting that tells the cell what passages of the book to read. These marks include chemical tags on DNA that make genes unreadable, as well as chemical tags on proteins that help expose DNA inside the cell nucleus, making genes readable. They’re the reason that cells from the liver, heart or brain differ profoundly. The National Institutes of Health Roadmap Epigenomics Consortium, including Kellis, published the most comprehensive map of the human epigenome in February.

Kellis led the data analysis team, which applied machine-learning algorithms to decode the language of the epigenome. The map served up important clues about how a single fertilized egg can develop into the diversity of tissues in the human body—and how healthy tissue can become diseased. For example, one team in the consortium reported how metastatic cancer cells contain an epigenetic fingerprint that reveals the tissue they came from, which could lead to better-targeted cancer treatments. Another team reported spotting DNA sequences that may trigger autoimmune diseases.

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Cracking Mutations’ Disease Code

Thousands of mutations are linked to diseases, yet only a few cause illness by directly disrupting proteins, the cell’s workhorses. Scientists have figured out how several of these mutations work.

Using thousands of tissue samples from cadavers, researchers on the Genotype-Tissue expression project (GTEx) isolated and sequenced DNA and a variety of protein-encoding RNAs from each sample. Many disease-linked mutations, it turned out, lie in stretches of DNA that don’t encode proteins themselves but instead regulate genes elsewhere in the genome that then go on to disrupt tissue function.

Genomic makeup also can affect how well a gene works, thereby causing disease, says Kristin Ardlie, a Broad Institute biologist who co-led the analysis published in May.
The Future of Primary Care

- Disease prevention
- Newborn/Fetal Screening
Currently screened conditions

- **PKU**
- **BIOT**
- **HCY**
- **MSUD**
- **GAL**
- **CH**
- **CAH**
- **CF**
- **SSD**
- **HIV**
- **TYR**
- **G-6-PD**
- **TOXO**

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Disease detection (PKU) and health promotion (PGX) on the same chip.
The Future of Primary Care

- Disease prevention
- Newborn/Fetal Screening
- Disease avoidance
- Genome/Exome characterization
Obesity

Eric Snyder et al.

The Human Obesity Gene Map: 2003 Update

Obesity Research 12(3): 369-439, March ’04

710 references, special populations

41 syndromes, fat distribution

Insulin, diabetes, growth hormone, lipin, leptin, leptin receptor, cholecystokinin receptor

450 genes, markers, chromosomal regions

23 chromosomes affected
The Future of Primary Care

- Disease prevention
- Newborn/Fetal Screening
- Disease avoidance
- Genome/Exome characterization
- Disease care
- Gene Insertion/manipulation
- Pharmacogenomics
Gene Therapy

Traditional
functional gene copy is added to replace
the defective or inactive gene

Pharmacologic
transmits a gene to produce:
  novel therapeutic agent
  toxic product to kill cells
Gene Therapy

Viral Vectors
- Retroviruses
- Adenoviruses
- Adeno- associated viruses
- Vaccinia
- Herpes simplex
- Lentiviruses
- Bacteriophage/ Plasmid
Gene therapy using an adenovirus vector
"In the very near future, primary care physicians will routinely perform genetic tests before writing a prescription because (they will) want to identify the poor responders."

F. Collins
(AAFP Annual Meeting, 1998)
Cat’s Claw

*Uncaria tomentosa*

- Anti-inflammatory
- NF-κ B inhibitor
- Scavenges free radicals

*Alim. Pharm. Ther.* 1998
*Free Radical Biology & Medicine,* 2000
Sangre de Grado

Antibacterial

*E. coli*

*Helicobacter sp.*

Inhibits neurogenic inflammation

blocks activation of sensory afferent nerves
The Future of Primary Care

- Disease prevention
- Newborn/Fetal Screening
- Disease avoidance
- Genome/Exome characterization
- Disease care
- Gene Insertion/manipulation
- Pharmacogenomics
- Counseling – Expanded Medical home
Genes

Nutrition

Cancers

Diseases
Ingredient Anxiety
Hyping what's not there
By Bonnie Hochman

Look closer in certain supermarket aisles and you might be baffled by what’s touted on the packaging. “Free of a.g. chemicals,” reads the label on one eco-friendly laundry detergent. A leading hair-care brand offers “sulfate free” shampoo. It’s easy to fall for a sigh of relief that ingredient anxiety is over.

But what’s wrong with sulfates? And how many products is it in? As grocery-store shoppers become more health conscious, many companies use high-litizing—often in big type—what their products don’t contain. Some of these non-ingredients, like BPA, have made headlines in recent years, while others are obscure that few consumers know much about them.

Bisphenol A (BPA)

People are saying, I don’t want products with fill-in-the-blanks,” says Cara Welch, chief scientist for the nonprofit Natural Products Association. Such complaints have led to a marketing misdirection, with some labels reflecting products that have been reformulated for the better and others doing little more than greenwashing. For instance, one liquid laundry detergent boasts on its label that it “contains no phosphates,” an ingredient the U.S. banned in laundry in 1995.

“These product claims can be tricky,” says Adam Lander, a senior analyst at the Environmental Working Group, an industry watchdog. “It’s hard for the average consumer to know if the ingredient they are avoiding doesn’t have exactly the one you would be most concerned about.” Welch advises the shorter the ingredient list, the better.

- Lithium
- Parabens
- Propylene glycol
- Sodium benzoate
- Sulfates
- Sulfites
Food for Thought
An Epigenetic Guide to Wellness
George J. Tobisch and Jo Anne Matar
We have more control than we think.