Epigenetics and Preconception

*The Ultimate Preventive Medicine*

Dr. Jaclyn Chasse, N.D.
What Causes Disease?:
Nature versus Nurture?

Genetics

Lifestyle
Meet the Genome

- Genetic changes can include:
  - **Mutations** (point mutations, deletions, duplications, etc)
  - **SNPs** (single nucleotide polymorphisms)
    - “tweaks” in metabolic pathway function
    - ie, MTHFR SNP
Meet the Genome

- For a long time, the paradigm in research was that genetics would provide understanding of the basis of disease
  - A gene (genotype) led to a certain physiology (phenotype)
  - With research, diseases would eventually be linked to their genetic origin
Meet the Genome

- “Genes can’t possibly explain all of what makes us what we are” – Craig Venter, President of Celera (the corporate side of the Human Genome Project)

By itself, a genome is passive- DNA can not make itself, assemble a protein, or perform a cellular function
Lifestyle Choices

Genetics

Lifestyle

Jaclyn Chasse ND, AANP 2011
Current Preventive Strategies

- Lifestyle modification
  - Encouraging physical activity
  - Healthy nutrition
  - Stress management
  - Smoking cessation
- Screening/Early Detection
Even with these strategies…
Incidence of Diagnosed Diabetes
2004

U.S. Center for Disease Control (www.cdc.gov)
Incidence of Diagnosed Diabetes
2005

U.S. Center for Disease Control (www.cdc.gov)
Incidence of Diagnosed Diabetes
2007

U.S. Center for Disease Control (www.cdc.gov)
Incidence of Diagnosed Diabetes
2008

U.S. Center for Disease Control (www.cdc.gov)
A Potential Decline in Life Expectancy in the United States in the 21st Century

S. Jay Olshansky, Ph.D., Douglas J. Passaro, M.D., Ronald C. Hershon, M.D., Jennifer Layden, M.P.H., Bruce A. Carnes, Ph.D., Jacob Brody, M.D., Leonard Hayflick, Ph.D., Robert N. Butler, M.D., David B. Allison, Ph.D., and David S. Ludwig, M.D., Ph.D.

SUMMARY

Forecasts of life expectancy are an important component of public policy that influence age-based entitlement programs such as Social Security and Medicare. Although the Social Security Administration recently raised its estimates of how long Americans are going to live in the 21st century, current trends in obesity in the United States suggest that these estimates may not be accurate. From our analysis of the effect of obesity on longevity, we conclude that the steady rise in life expectancy during the past two centuries may soon come to a similar method but different assumptions to arrive at a projected life expectancy of 100 years for males and females in most countries by the year 2300.7 The Social Security Administration (SSA) arrived at a more tempered but still optimistic view that life expectancy in the United States will continue its steady increases, reaching the mid-80s later in this century.8

A recently convened panel of advisers,9 and some mathematical demographers who advocate the use of extrapolation,10 have advised the SSA to project an even more rapid rate of increase in life expectancy for the U.S. population beyond that already anticipated by the SSA.11
What are we missing?
Epigenetics

Describes heritable changes in gene function that do not involve a change to the genome
Epigenetic networks are dynamic. Genes code for proteins and those proteins have biochemical reactions and reaction products. This result can then alter genetic expression in order to optimize a cell’s function in its own environment.
How do we address epigenetic influence?

- Lifestyle choices
- Targeted nutritional therapies
- Development of pharmaceuticals
  - Azacitidine (inhibitor of DNA methylation) for myelodysplastic syndromes
How do epigenetic changes happen?

- DNA Methylation
- Histone acetylation
- Modification of DNA function by RNA
  - siRNA
  - miRNA
DNA Methylation

- Adds a methyl group to CpG dinucleotide
- Usually in promoter regions of genes
- Affects binding of methyl-sensitive DNA-binding proteins
- Influences conformation of chromatin
TWO WAYS TO ACTIVATE OR SILENCE GENES

1. Methylation
   - DNA methylation
     Methyl marks added to certain DNA bases repress gene activity.

2. Small molecules/histones
   - Histone modification
     A combination of different molecules can attach to the "tails" of proteins called histones. These alter the activity of the DNA wrapped around them.

   - (Folic acid, Vitamin B12, Choline)

   - Resveratrol, quercetin, curcumin, other small molecules (histone deacetylase inhibitors)
Methylation and Nutrition

- Methyl groups required for all methylation reactions in humans
- This one-carbon metabolism is highly dependant on dietary methyl donors and cofactors
Methylation and Nutrition

<table>
<thead>
<tr>
<th>Methyl donors</th>
<th>Cofactors in methyl metabolism</th>
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<tbody>
<tr>
<td>Methioinine</td>
<td>Folic Acid</td>
</tr>
<tr>
<td>Choline</td>
<td>Vitamin B12</td>
</tr>
<tr>
<td></td>
<td>Pyridoxyl Phosphate</td>
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</table>
Epigenetics

- Can be influenced by environmental factors, nutrition, supplementation!
- Doesn’t change the book, but changes how it is read!
The Agouti mouse

Mothers fed the same chow during pregnancy.

No supplementation

Supplementation with
- Folic acid
- Choline
- Anhydrous betaine

Mothers fed the same chow during pregnancy.
Agouti Mice

Mother of brown mouse was supplemented 2 weeks before mating through pregnancy and lactation. After weaning, mice fed the same chow for 21 days, when the picture was taken. Methylators given to mother changed genetic expression of offspring.
Overkalix Study

- Followed 303 men and women born in 1920.
- Looked at their parents’ and grandparents’ food supply during the prepubertal slow growth period (SGP, ages 8-12 years)
- Study found that changes in nutritional status of the parents and grandparents during the SGP modified disease risk in the subjects
“Transgenerational responses to ancestors’ nutrition prevailed as the main influence on longevity.”
Current Preventive Medicine practice

- Focus on patients’ own behavior in a one-dimensional way
  - Stress management
  - Diet
  - Environmental exposures
  - Exercise

- We are missing out on the opportunity to make a greater epigenetic impact!
Key Periods of Imprinting/Impact

- Preconception
- Fetal developmental period
- Puberty
Key Periods of Imprinting/Impact

- Preconception (especially for women)
  - Maturation of gametes

- Fetal developmental period
  - Stem cell differentiation
  - Rapidly dividing cells are very susceptible to changes in their environments
  - In girls, development of gametes/eggs

- Puberty
  - Time of maturation of spermatogenic organs, especially in males
Preconception
Preconception

- For both men and women, maturation of egg and sperm happens prior to conception
- 4 month window of maturation
- Quality of gametes can depend on
  - Nutritional status
  - Environmental exposures
  - Stress
Why increased risk at preconception?

Reprogramming erases most epigenetic tags so that the fertilized egg can develop into any type of cell.

Reproductive cells
- Specialized function
- Lots of epigenetic tags

Male and female reproductive cells join
- Can become any type of cell
- Few epigenetic tags

Embryo develops
- Many cells with specialized functions
- Lots of epigenetic tags
Preconception

• Once fertilization occurs, genome undergoes extensive demethylation.
  • Allows for potential of all genes to be transcribed
• Cytosine methylation is re-established after implantation
• DNA methylation must then be maintained throughout many rounds of rapid cellular proliferation

• Early methyl donor malnutrition (over or under) can lead to epigenetic changes impacting disease
Essential nutrients during Preconception

Epigenetic Influence
- Folic Acid
- Vitamin B12
- Iron, Zinc

Protection from Oxidative Damage
- Vitamins A, C, E, Zinc, Selenium

Other
- Omega 3 fatty acids

Prenatal Influences
Perinatal influences

- Nutritional factors
  - Maternal food supply: feast and famine
  - Maternal macronutrient intake
  - Gestational blood sugar
  - Nutrient-specific interactions
- Environmental factors
  - PACs and birth outcomes
  - Phthalates
  - Xenoestrogens
- Emotional factors
  - Stress and anxiety
The Barker Hypothesis

- People who had a low birth weight are at greater risk of developing coronary heart disease
- Initial study looked at death rates in 100,000 men and women in Herpshire, UK from 1911-1930.
- Similar studies completed on almost every continent

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<thead>
<tr>
<th>Subjects</th>
<th>Number of Subjects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danish men and women</td>
<td>210,662</td>
<td>Epidemiology 2008;19:197-203.</td>
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</table>
Birth weight and Heart Disease

- Birth weight was inversely correlated with early death secondary to coronary heart disease
- Higher birth weights also appear to have consequences
- May be due to mismatching in prenatal and postnatal life

Low Birth Weight

- Birth weight is not determined by genetic variation, but by prenatal environment.
  - Intrauterine environment may place constraints on a growing fetus
    - Small uterine size
    - Nutrient availability
    - Oxygen supply
    - Hormonal exposure

Low Birth Weight

- Low birth weight also correlated with higher incidence of childhood asthma and onset of acute leukemia in children
- Low volume of placenta has also been associated with increased cardio-metabolic risk

High Birth Weight

- High birth weight has been correlated with higher incidence of breast cancer, hepatoblastoma

Maternal Malnutrition

- Severe famine in Netherlands November 1944- May 1945
- Daily rations of food during Nazi blockade were 400-800 kcal
- May 1945, famine ended and rationing stopped. Normal eating returned.
Dutch Famine

- Exposure to famine increased offsprings’ risk of
  - Obesity
  - Mood disorders
  - Impaired glucose metabolism
  - Impaired lipid metabolism
    - Hypercholesterolemia
  - Reduced renal function
  - Hypertension

Risk varied depending on gestational age at time of famine.

Early Hum Development 2006;82:485-91
Dutch Famine

- After 60 years, those conceived during the famine had less methylation than unexposed same-sex sibling on marker genes
- Global nutrient restriction also demonstrated alterations to HPA axis
  - Decreased production of ACTH and cortisol after administration of CRH
  - Decreased cortisol response to ACTH

Maternal Nutrition and Schizophrenia

- Prenatal famine doubled rates of schizophrenia in offspring in Netherlands and China.

Maternal Low Protein Diet and Blood Sugar

- Maternal low protein diet correlated with
  - Increased glucocorticoid receptor (GR) expression
    - Leads to increased capacity for gluconeogenesis, which may contribute to insulin resistance
  - Decreased expression of enzyme that inactivates glucocorticoid receptor (11B-hydroxysteroid dehydrogenase type II)
  - Upregulates glucokinase (GK) expression in liver
    - Increased capacity for glucose uptake

Maternal Low Protein Diet and Blood Sugar

- Promoter genes imprinted for glucocorticoid metabolism
- Increased gene transcription leads to increased susceptibility of metabolic syndrome phenotypes.

Maternal Low Protein Diet and Lipid Balance

- Maternal low protein diet correlated with
  - Increased expression of Acetyl Co-A carboxylase and fatty acid synthase in liver.
  - Increased blood triacylglycerol (TAG) and fatty acid concentrations.
  - Impaired lipid metabolism.

- Lower concentrations of DHA in liver and brain
  - In non-supplemented diet, so dependant on conversion from a-linolenic acid

Br J Nutr 2003;90:345-52
Maternal Low Protein Diet
Epigenetic effects

- Vascular dysfunction
- Impaired immunity
- Increased susceptibility to oxidative stress
- Increased fat deposition
- Altered feeding behavior

These effects can be decreased/prevented with maternal supplementation of folic acid!!

Nutr Res 2000;20:995-1005
Mech Ageing Dev 2005;126:804-12
Maternal High Protein Diet

- Motherwell, Scotland study
- Mothers instructed to consume 0.45 kg meat daily and to avoid carbohydrates during pregnancy
- Adult offspring had
  - Increased cholesterol
  - Increased blood cortisol levels

J Clin Endocrinol Metab 2003;88:3554-60.
Potential nutrient intervention

- Folate (5 mg/kg or 150 mg dose for adult)
- Glycine (5 mg/kg or 150 mg dose)
- Butyrate
- Sulforaphane
- Garlic organosulfur compounds
- Zinc
- Iron
- Vitamin D
- Niacinamide
- Riboflavin
- Vitamin B12
- Vitamin A

Prenatal imprinting

- Maternal dietary exposure to methyl donors (folic acid, SAM) may be a determinant in modulating the susceptibility to diseases in adult life
  - Diabetes
  - Metabolic syndrome
  - Glucocorticoid excess
  - Obesity
  - Hypertension
  - Insulin resistance
  - Hyperlipidemia
  - Hyperglycemia

Environmental Exposures during pregnancy

- Direct toxicity
- Impact on epigenetics and gene expression
- Can impact future generations
BPA exposure during pregnancy

- Pregnant agouti mice were exposed to BPA
- Offspring developed into yellow, obese mice more frequently than expected.
- DNA Methylation at the agouti gene sites (as well as others) was decreased by 31%
- Methylation was consistent throughout the mouse’s body, suggesting demethylation occurred in early development
- BPA was demonstrated to remove methyl groups from DNA
BPA exposure during pregnancy

- Further studies have demonstrated that co-exposure to certain nutrients were protective
  - Folic Acid
  - Vitamin B12
  - Genistein from soy

Xenoestrogen exposure during pregnancy

- Exposure of pregnant rats to alkylphenol polyethoxylates like OPP, and phthalates like BPP reduced mean testicular size in offspring
- Morphology normal
- Sperm count decreased 10-21% in offspring
- Octylphenol exposure suppressed FSH secretion, testis size and sertoli cell number

Environmental Health Perspectives 1995;103(12):1136.
Endocrinology 2000;141(7):2667.
After transient embryonic exposure, adult animals from the F1 generation and all subsequent generations examined (F1-F4) developed a number of disease states or tissue abnormalities...
Prostate disease  
Sperm abnormalities  
Kidney disease  

Immune dysfunction  
Hypercholesterolemia  
Testis abnormalities
Lead exposure and Alzheimer’s

- Exposure to lead during prenatal development period in rats led to an increased production of
  - APP (Amyloid Precursor Protein)
  - Abeta
  - Oxidative marker 8-oxo-dG

In the aging brain

- Pathogenesis not observed in younger brain, suggesting epigenetic changes and influence on gene transcription and oxidative DNA damage

J Mol Neurosci 2008;34(1):1-7..
Polycyclic Aromatic Hydrocarbons (PAH)

- Prenatal exposure of PAH in air/ cigarette smoke have been linked with
  - Increased measures in cord blood
  - Poor behavior scores in NYC children
  - Developmental delay
  - Low birth weight, chest and head circumference
  - Asthma

Should we be concerned?

- EWG assessed adult volunteers with no known toxic exposure (n=9):
  - 167 chemicals (average 91) were found in blood and urine of study participants
- EWG study on newborn cord blood (n=10)
  - Average of 200 industrial compounds, pollutants, and other chemicals
    - Included DDT, dieldrin, perfluorochemicals, brominated fire retardants, PCBs, PAHs, dioxins, furans, polychlorinated napthalenes, and mercury
Should we be concerned?

- CDC reported urinary phthalates and their metabolites in over 75% of study participants’ urine
- BPA was detected in more than 95% of adults studied (n=400)

www.cdc.gov
Protection from Environmental Pollutants

- Some nutrients have demonstrated protective effects from environmental exposures
  - Vitamin A taken pre-pregnancy has demonstrated reduction in risk of adverse birth outcomes due to prenatal pollutant exposure in Poland.
- Further studies have demonstrated that co-exposure to certain nutrients were protective
  - Folic Acid
  - Vitamin B12
  - Genistein from soy

Maternal stress during gestation

- Maternal stress during gestation has demonstrated
  - Alterations of HPA axis in offspring
  - Alteration in brain neurotransmitter systems
  - Increased anxiety in offspring
  - Increase emotionality
  - Decreased motor development
  - Decreased learning abilities
  - Decreased length of gestation
  - Decreased birth weight (and associated effects)

Kofman 2002
Maccari et al 2003
Welberg et al 2001
Maternal stress and immunity

- Maternal stress during gestation has demonstrated
  - Decreased macrophage and neutrophil function in 2 month old offspring
  - Decreased cytotoxicity of NK cells
  - Decreased production of TNF-a and IL-6 upon stimulation with LPS (lipopolysaccharide) in rhesus monkeys
  - Increased corticosterone and fever response to LPS in rats.
  - Decreased thymic size and function

Maternal stress and immunity

Maternal stress and atopic disease

- Maternal stress during gestation has demonstrated
  - Increased risk of atopic diseases
    - Self-reported high anxiety in pg associated with increased IgE in cord blood
  - Increased level of pro-inflammatory cytokines in mother
    - Increased exposure to pro-inflammatory cytokines in utero is suspected to increase risk of allergy for infant later in life

Maternal stress and offspring stress response

- Maternal depression and anxiety in 3rd trimester led to offspring with increased methylation of glucocorticoid receptor NR3CI gene
- This was associated with increased salivary cortisol stress response in infants at 3 months.
- Response not blunted in infants born to mothers treated with SSRIs

Epigenetics 2008;3(2):97-106
Maternal nurturing and epigenetics

- Studied maternal behavior during suckling
  - Pups from mothers with decreased nurturing demonstrated an increased stress response
  - Due to hypermethylation of nucleotides in promoter region of GR in hippocampus of the offspring.
  - Pups of mothers with increased nurturing demonstrated a better response to future stress.
Assisted Reproduction and Epigenetic Impact

- There is a demonstrated difference in DNA methylation patterns in samples from children born after IVF vs children conceived naturally
  - Ovarian stimulation leads to elevated follicular homocysteine levels
  - Embryo culture medium is usually amino acid free, which means no exposure to methionine in the first 3 days of in-vitro culture, a time when methylation is very important!

Key Prenatal Interventions

- **Good nutrition**
  - Avoidance of over or under nutrition
  - Balanced diet with moderate protein intake (1-1.5 g/kg)
  - Supplementation with nutrients with demonstrated safety and benefit in prenatal period

- **Avoidance of exposure to environmental pollutants**

- **Stress Management**
  - Prenatal yoga
  - Slowing down of lifestyle!
Pubertal

- Slow growth period (Ages 8-12)
- Some effects seem to be sex-specific
  - Avon Longitudinal Study
    - Fathers who started smoking before age 11 years had sons (but not daughters) with greater BMI
    - Paternal grandfather’s food supply associated with mortality of grandsons only, grandmothers’ food supply associated with mortality of granddaughters

Eur J Hum Genetics 2006;14:159-66.
Pre-Pubertal Behaviors
Pubertal Nutrition

- Several Studies with similar results
  - Males had increased risk of mortality if their fathers had good nutrition during their slow growth period (SGP)
  - When a father experienced poor food availability or famine during the SGP, their sons were protected against cardiovascular death
  - Similar trends found in grandsons and granddaughters of grandfathers and grandmothers (respectively) who had plentiful food supply during their SGP, although not as pronounced.
  - With mothers, protection was conferred to female offspring when mother had plentiful food during her SGP

Pubertal Food Supply

During slow growth period, food availability has an impact on transgenerational response

“Transgenerational responses to ancestors’ nutrition prevailed as the main influence on longevity.”

Eur J Hum Genetics 2006;14:159-66.
Pubertal Nutrition
Interventions during Puberty

- Focus on nutrition during this time, especially among boys ages 8-12
- Education about impact of lifestyle habits such as smoking and poor diet on self and future generations
- Consider supplementation with basic nutrients such as multivitamin and omega 3 to support healthy gamete development.
Conclusions

- Origin of adult disease is multifactorial
- Current medical paradigm focuses on interventions for an individual to impact their own health
- Inadequate concentration on the impact of parental epigenetic and environmental origins of disease
- Increased focus on prevention is needed during preconception, perinatal, and pubertal times
I hope you enjoyed the webinar!

If you would like to set up an Emerson account, please contact

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