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Are Health Problems in Adulthood linked to our Experiences in the Womb? An Epigenetic Approach

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Abstract

The origins of adult disease have been a prime topic for research, as deciphering causes can lead to strategies for preventions and cures. There has been recent intrigue focused on the environment in the womb. Records from England and Wales in 1911 show that those who suffered from cardiovascular disease were geographically correlated with regions high in infant mortality in the past, seventy years before the study. When looking into the cause of the neonatal death rates, low birth weight, poor maternal health, and high maternal death rates during childbirth were clearly associated. Barker inferred that there is much happening in the intrauterine environment that ultimately affects our quality of life. This research helps support the concept that rather than looking at childhood adversity and socioeconomic circumstances to help explain adult disease, we might need to reach further back (Barker, 1990).

Through epigenetic mechanisms, alterations to the physical expression of our DNA can take place without changing the sequences of the actual DNA. Epigenetics is what is responsible during fetal development for the differentiation of stem cells to specific cells by adding or subtracting methyl groups to silence or activate particular genes. When an egg and sperm unite all previous methylation patterns are stripped from the newly formed diploid. However, by the time it becomes a blastocyst, new patterns have already formed. It is during this crucial stage of development that new patterns and changes to our epigenome our founded and passed down (Powledge, 2009).

The purpose of this paper is to determine if chronic health issues in adulthood have their roots in the environment and changes experienced by the fetus in the womb. Environmental exposures like nutrition, stress, and toxicants are evaluated and tested for their potential hand in setting the course for adult disease. Many studies show correlation between malnutrition and the development of metabolic diseases like obesity, type II diabetes, and even hypertension. Likewise, stress during gestation has been linked to anxiety, depression, and posttraumatic stress disorder (PTSD) in offspring. Toxicants like bisphenol-A are the likely culprit for genetically expressed abnormalities that range from cancers of the reproductive system to attention/deficit hyperactive disorder (ADHD) in exposed offspring. The "how" and the "why" are explored in this paper. If we can better understand the origins of adult disease then we are better equipped to defend our future generations against it. Furthermore, the notion that our DNA is not at fault for these outcomes, but rather epigenetic adjustments written on top of our DNA, provides hope that just as easily as it can be added on, we can take it off (Powledge, 2009).

Introduction: What is Epigenetics?

The concept that the in utero environment causes adult diseases later in life was first proposed by DJ Barker in the famous, "Barker Hypothesis," some twenty years ago, but the term, "epigenetics," was originally coined by developmental biologist Conrad Waddington in the early 1940s. Waddington labelled it as, "the interactions of genes with their environment, which brings the phenotype into being." He was referring to the phenomenon that all cells share the same DNA, yet they express complete differences in their phenotype and function. Moreover, skin cells, liver cells, and brain cells are only capable of producing more of their type within their same phenotypic expression, so whatever mechanism is accounting for the differentiation is clearly able to be inherited. These modifications usually take place on certain regions of DNA, cytosine-phosphate-guanine dinucleotides, at the carbon-5' position of cytosine. The most common mechanism seen in the fetal environment includes methylation, the adding or removing of a methyl group to and from the carbon-5' position. The usual methyl donor is S-adenosylmethionine (SAM), which the methyl transferase uses as a source of methyl to the DNA molecule. During methylation, methyl-transferase adds on the methyl group, preventing binding of transcription elements, thereby silencing gene expression (figure 1) (Odom, Taylor, 2010).

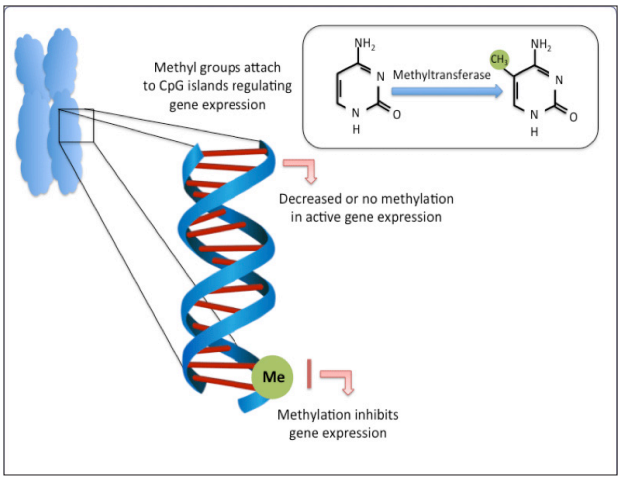

Figure 1

The silencing of genes via methylation occurs by obstruction of the binding of transcriptional machinery that needs contact with the cytosine, in the major groove of the double helix. Methylation can also function by methylating the promoter region of DNA. Methylation can sometimes lead to activation of a gene by obstructing the binding of transcription repressors, ultimately activating certain genes. Another common mechanism involves post translational histone modification, with the

addition or subtraction of different compounds like methyl, acetate, or phosphate groups to histone proteins, causing changes in chromatin packaging. The adding on of these compounds to the four pairs of proteins that make up the "octameric histone core…restricts physical access of nuclear factors to the DNA and alters gene expression" (Odom, Taylor, 2010).

Methylation happens at very critical times in development. During gametogenesis, haploid cells demethylate from the development of primordial germ cells until the period before preimplantation, to return the cell to its pluripotent state. The pluripotency refers to the cells ability to take the shape and responsibility of any cell type in the body. After implantation of the embryo, remethylation begins to differentiate each cell, forming cell specific DNA methylation patterns (Odom, Taylor, 2010).

Methylation and histone modification are seen as adaptive responses. In other words, it is a mechanism to protect the fetus from the volatile environment in which it is developing. For example, in a situation of food shortage, metabolic adaptations are made to increase the energy storage. Problems arise, however, when the adult environment does not match the environment in the womb. When the fetus is subsequently born into a nutrient-rich environment, suddenly its metabolic adaptations can lead it on a path of obesity and all its associated medical complications. (Odom, Taylor, 2010) It is with this idea that we construct a hypothesis that links adult chronic disease to in utero environmental exposures.

Methods

This comprehensive review is based on critical analysis of literature on epigenetics obtained using the Touro College Online library and PubMed publishing, analyzing the information and opinions that were expressed in various experiments. Review articles were useful in providing additional references to source material.

Discussion: Nutrition

Malnutrition

Nutrition is extremely significant when looking at studies to link fetal environment to adult disease. The majority that is known on the epidemiology of epigenetics comes from animal studies, yet the Dutch Famine of 1944-1945 affords a unique opportunity to study the effects of malnutrition on a human population. The period of the famine was clearly defined, the food rations were documented, and registries and health care documentation remained intact. (Heijmans et al., 2008)

Insulin-like growth factor II (IGF-2) is one of the most epigenetically controlled loci. IGF-2 is integral for human growth and development, is known to play a significant role in "metabolic regulation of glucose homeostasis, cardiovascular functions, and lipid metabolism,"(Rijlaarsdam et al., 2016), and is maternally imprinted. This means that while both alleles are inherited, the maternal allele is silenced via methylation. If hypomethylation, the decrease of methylation via subtracting of methyl groups, takes place in these regions, bi-allelic expression of IGF-2 occurs, leading to detrimental outcomes in adult health. (Heijmans et al., 2008)

A previous study involving 372 twins shows that the methylation of IGF-2 largely depends on genetic factors and has methylation marks that are stable at least up until middle age (Heijmans et al., 2008). Therefore, any alterations from the genetic disposition in the methylation patterns done during gestation would be able to be detected many years later. The many different diseases that have been linked to the Dutch Famine are thought to be mediated by abnormal methylation of IGF-2.

A study was done to test methylation patterns of children conceived during The Dutch Famine in comparison to their same sex siblings. The study was careful to segregate those that experienced famine in the periconceptional period and those that experienced famine during late gestation. Blood was drawn from the 122 subjects and their same sex siblings, treated with bisulfite to segregate methylated regions of IGF-2, and then amplified using polymerase chain reaction (PCR). The region contained five CpG islands, areas that are rich in cytosine-phosphate-guanine dinucleotides. In the 60 adults whose mothers experience the famine in early gestation, all but one island showed hypomethylation of between 5-6%. Interestingly, those that were exposed to the famine during late gestation did not have differing methylation patterns than their siblings, suggesting that methylation pattern of the IGF-2 allele are susceptible to environmental influences at a critical window in early development (figure 2). Those exposed during late gestation did exhibit lower birth weight than the other groups, a birth feature that was previously associated with adult disease. This study shows that while low birth weight might be indicative of malnutrition, it is epigenetic modifications that are thought to hold responsibility for deteriorating adult health. (Heijmans et al., 2008)

Coronary artery disease (CAD) is strongly linked to fetuses exposed to the Dutch Famine. Developing organ systems respond negatively to a lack of nutrients available, especially during periods of critical development. Using a registrar of 2,414 infants from the Dutch Famine Birth Cohort born between the dates of November 1, 1943 and February 28,1947, Painter et al. extracted a group of 975 participants that experienced a caloric intake under 1,000 calories for at least 13 weeks of gestation during the famine. Various medical information was collected from participants at age 50 and 58, including blood pressure, glucose tolerance test results, total cholesterol levels, and electrocardiogram information. (Painter et al., 2006)

By the end of this study, 83 candidates developed coronary artery disease, with the highest cumulative incidence of coronary artery disease at 13% seen in those exposed during early gestation. Interestingly, it was those born in mid and late gestation that were born with the lowest birth weights with mothers

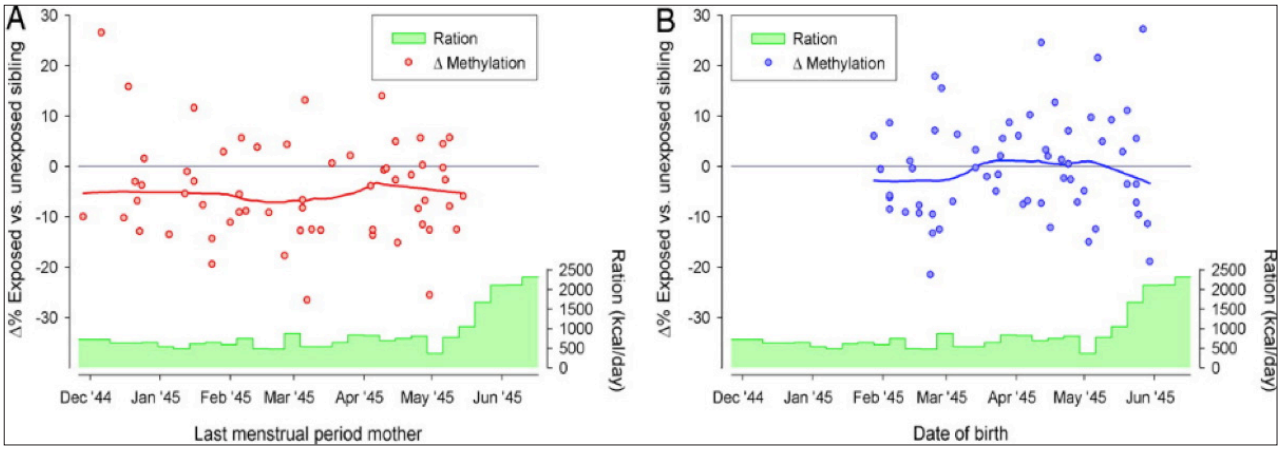

Figure 2, showing different methylation patterns between those exposed periconceptionally and those exposed in late gestation (Heijmans et al. 2007)

that weighed the least. Yet, those that developed coronary artery disease after exposure in mid to late gestation did not show a significant difference in cumulative incidence compared to those that were unexposed. On average, coronary artery disease manifested three years earlier in adults exposed in early gestation than in persons unexposed. (Painter et al., 2006) These results support the previous study that hypomethylation affects fetuses exposed to lack of nutrition in early gestation, thus linking hypomethylation of IGF-2 to adult disease as opposed to small birth weight. (Heijmans et al., 2007) That being said, any exposure to famine correlated with elevated glucose concentrations at 120 minutes and elevated ratios of LDL (low density lipoproteins) to HDL (high density lipoproteins). Most of the subjects were also being treated for type II diabetes and hypocholesterolemia (Painter et al 2006).

Schizophrenia is also linked to this critical window. A cohort of persons born in cities in western Netherlands during 1944-1946 were compared for their risk of schizophrenia. The study included those exposed in early gestation compared with those exposed at other points of pregnancy. A national psychiatric registry of the region was referenced to see the frequency of patients hospitalized at age 24 to 48 years in the two groups of subjects. The results attested to the fact the those conceived at the height of the famine (i.e., that were exposed in early gestation) had a statistically significant twofold increase in the risk for schizophrenia (Susser et al., 1996).

High-Sugar-High Fat Diet

High fat high sugar diets are associated with development of ADHD, conduct problems (CP), and their co-occurrence. IGF-2 may also be linked to ADHD, as it is a major modulator of placental and fetal growth, as well as brain development after birth. Previous animal studies have linked IGF-2 to developmental abnormalities in structure and/or function of the cerebellum and hippocampus, two regions that play significant roles in ADHD. As previously noted, IGF-2 is easily influenced by diet during critical periods of development (Heijmans et al., 2008). Studying a sample of cohorts of mothers and 164 children with early onset conduct problems (EOP) or low conduct problems (CP), researchers considered the link between prenatal diet, IGF-2 methylation status, current conduct problems, and risk symptoms for ADHD (Rijlaarsdam et al., 2016).

DNA methylation information was extracted from cord blood of the children from birth and peripheral blood at age seven. It was then bisulfite converted using the EZ-DNA methylation kit. A large number of probes, 139, were extracted that are mapped to IGF-2 or overlapping regions, with methylation at factor 1 the most relevant to the study. Researchers assessed the mothers' diets during pregnancy via The Food Frequency Questionnaire (FFQ), recording the maternal dietary patterns at 32 weeks of gestation and child's diet at age 3, 4.5, and 7 years of age. The FFQ recorded the frequency of consumption of particular foods with higher frequency indicated by higher scores. High scores in processed foods (chips, fried foods, pasties) and confectionery foods (chocolate bars, cakes, biscuits) indicated a prenatal and postnatal high-fat and sugar diet. At three different occasions (age 7, 10, and 13), ADHD symptoms were assessed with the Development and Well-Being Assessment (DAWBA), a semi-structured interview with open and closed questions directed toward parents about a range of symptoms seen in their children relevant to ADHD, oppositional defiant disorder (ODD), generalized anxiety disorder (GAD), conduct disorder (CD), and major depressive disorder (MDD) (Rijlaarsdam et al., 2016).

Results from this study indicate that youth with early onset persistent conduct problems (n= 83) exhibited higher levels of ADHD symptoms than youth with low conduct problems (n=81). The study also found that early onset persistent conduct problems youth showed high factor 1 methylation at birth of IGF-2 with symptoms of ADHD, but at age 7, methylation levels were negatively correlated with postnatal cumulative risk, with risk domains including life events such as relative's death, contextual risk like financial problems, or direct victimization like

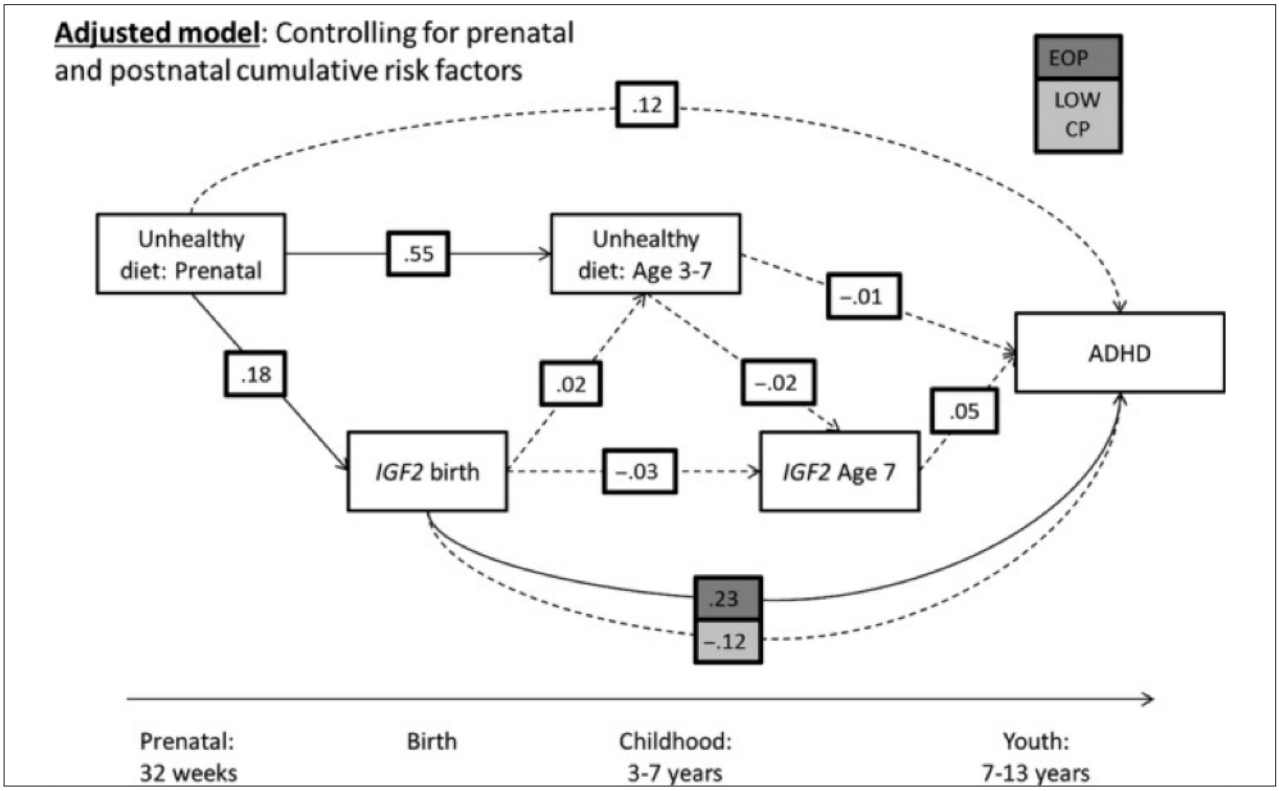

Figure 3, "Prospective interrelationships between unhealthy diet, IGF2 methylation and ADHD for youth with early-onset persistent (n = 83) versus low (n = 81) conduct problems. Multiple group path analysis. Solid arrowed lines indicate standardized path coefficients that survived bootstrap-corrected confidence intervals (i.e. significant paths) for EOP versus low CP youth or averaged across all youth" (Rijlaarsdam et al., 2016).

bullying. This can be seen as further proof that the prenatal environment is the true origin for postnatal disease development, as opposed to what the child experiences after birth. IGF-2 methylation did not correlate with ODD, GAD, or MDD symptoms (Rijlaarsdam et al., 2016).

Cross-lagged associations showed that prenatal diet was associated with IGF-2 methylation at birth for both early onset persistent conduct problems and low conduct problems. However, associations between methylation at birth and ADHD symptoms at ages 7-13 was significantly higher in early onset problem youth than in low conduct problem youth, even though the maternal diet was significant in both domains (figure 3). In early onset persistent conduct problems youth, high methylation did predict ADHD symptoms, linked through the epigenetic modifications that were caused from unhealthy eating habits. It is possible that this vulnerable developmental pathway presents a specific risk factor for EOP children only (Rijlaarsdam, et al., 2016).

Methyl-Supplements

Methylation, the addition of methyl groups to cytosine-phosphate-guanine dinucleotides or to histone proteins, is what causes much of the gene silencing that takes place during fetal programming and early development. For methylation to happen, methyl donors like the B vitamins are vital in our diets. Methylation frequently occurs at transposable elements, DNA sequences that are unique in their ability to "jump" positions on the genome. At first glance, they seem rather parasitic, the source of genetic diseases and mutations by a series of rearrangements and recombination. However, mobile DNA has recently been discovered to have an important role in biodiversity and evolution. A concept known as "molecular domestication" claims that open reading frames, complete RNA, and coding exons are thought to have originated from transposed DNA (Bohne et al., 2008). When methylation patterns differ on transposable elements, they account for epigenetic "mosaicism" and varying phenotypes. They are likely the cause of differential gene expression in identical twins. These regions are also metastable and therefore, very susceptible to nutritional influences (Waterland and Jirtle, 2013).

To analyze the effect methyl donors might have on the developing epigenome, yellow agouti mice were used. The murine agouti gene encodes for a paracrine signaling molecule that instructs follicular melanocytes to switch from black eumelanin production to yellow phaeomelanin, resulting in yellow fur. This is known as the agouti A allele with the pseudoexon 1A pointing away from the agouti allele. The agouti "a" allele is caused from

a loss-of function mutation in A, causing homozygotic "a" allele mice to be black. In contrast, the Avy allele is caused by the insertion of an intracisternal A particle (IAP) retrotransposon to the 5' end of the A allele, within the agouti exon 1A. This ectopic gene is transcribed from a cryptic promoter in the proximal end of the intracisternal A particle (figure 4). The CpG methylation of this region varies largely among different mice, inversely correlated with ectopic agouti expression. This variability is also responsible for a wide variation of hair color, adiposity, glucose tolerance, and tumor susceptibility in Avy/a mice that are otherwise isogenic (Waterland and Jirtle, 2003).

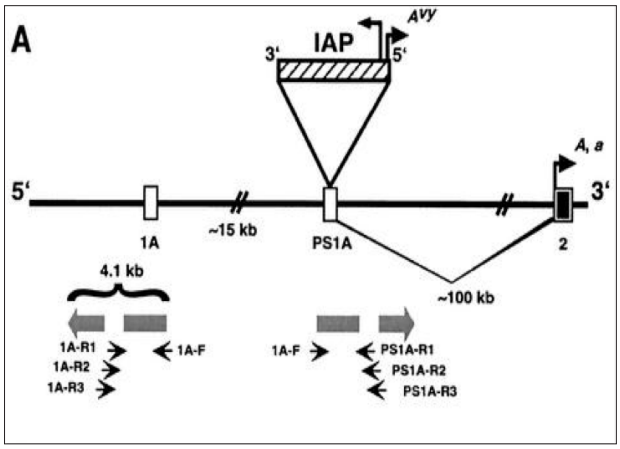

Figure 4, "IAP insertion site in Avy allele. (A) Exon 1A of the murine agouti gene lies within an interrupted 4.1-kb inverted duplication (shaded block arrows). The duplication gave rise to pseudoexon 1A (PS1A)." (Waterland and Jirtle et al., 2003)

If a/a dams are given specific dietary supplementation before mating with Avy mice, their Avy/a offspring exhibit a shift in coat color from yellow to pseudoagouti brown. Because the coat color correlates with Avy methylation status, it is inferred that supplementation influences this phenotypic shift via methylation of the Avy gene. To test this hypothesis, a population of congenic Avy mice that had been sibling mated for forced heterozygosity for 200 generations were prepared. In another group, a/a female mice that were 8 weeks old were randomly assigned to either NIH-31 diets or NIH-31 diets supplemented with methyl donors and cofactors like folic acid, vitamin B12, choline chloride, and anhydrous betaine. The diet was issued for two weeks before mating with the Avy mice, as well as throughout pregnancy and lactation. At age 21 days, the Avy/a offspring were weighed, tail tipped, photographed, and evaluated for coat color of yellow, slightly mottled (a bit of brown mixed in with yellow), mottled (half yellow, half brown), heavily mottled (greater than half is brown), or pseudoagouti. Pseudoagouti refers to the silencing of ectopic agouti expression, causing the brown agouti phenotype of an A/- mouse (Waterland and Jirtle, 2003).

The supplemented dams did in fact express the brown color of pseudoagouti phenotype. Using tail tip DNA, researcher quantified the result from seven different CpG sites of the region, showing methylation at each site, whereas the non-supplemented dams seemed to have methylation distributed bimodally with the epigenetic switch in one of two different states. This observation further proves the notion that methylation of Avy is the mediator of the affects of nutrition on coat color (figure 5). Furthermore, methylation patterns in the tail correlated with methylation patterns in the liver, kidney, and brain, representing the early embryo layers of endoderm, mesoderm, and ectoderm, respectively. This continued to show that such methylation patterns were created at the earliest stages of embryo development and were maintained throughout development (Waterland and Jirtle, 2003).

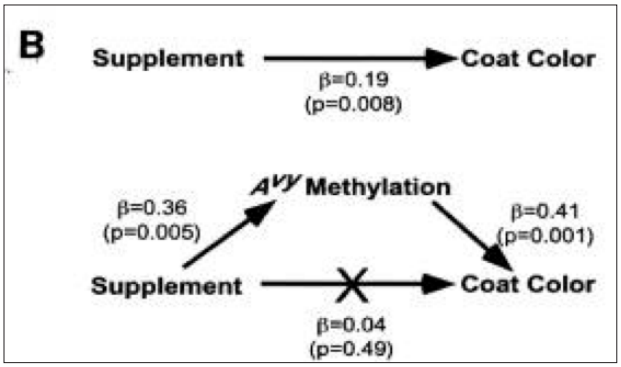

Figure 5 shows that once adding methylation to the model, no correlation was significant between supplement and color alone. Methylation was the clear mediator.

Forty percent of the human genome is made of transposable elements, with copying number exceeding double the magnitude of classical genes. Much variation can be made by simple recombination and transposition of the elements in different areas. Epigenetic modification is likely in these areas (Bohne et al., 2008). Furthermore, many human genes are also transcribed from a cryptic promoter, similar to how the ectopic agouti transcription originates from the Avy intracisternal A particle. This study provides compelling evidence that much of our own phenotypic variability and "epigenetic mosaic" can be influenced by early nutrition (Waterland and Jirtle, et al., 2003).

The restriction of methyl donors has previously been linked to neural tube defects in infants. Pregnant women are careful to take folic acid to avoid this fatal outcome. However, the fetus's epigenome is so sensitive, that even exposure like slightly improper methyl donor levels can bring about its own set of variations. Sinclair et al. experimented by restricting specific B vitamins and methionine within the normal physiological range to explore its effect on adult offspring. He administered a specific diet to adult female sheep from six days before conception until eight weeks after conception. Diets were formed containing all nutrient requirements with decreased vitamin B and sulfur amino acids, yet within the physiological range in both

sheep and human to be considered non-toxic. Because gametes and preimplantation embryos go through extensive methylation reprogramming, this experiment was used to test, "the extent to which periconceptional availability of methyl groups altered DNA methylation of gene-associated CpG islands and affected adult health status" (Sinclair, et al., 2007).

Twenty five ewes that were placed on the 'methyl deficient' (MD) diet showed homocysteine levels in follicular fluid, plasma, and in granulosa cell lysates higher than the 25 ewes that were in the control diet with no methyl deficiency. Most importantly, the incorporation of methionine into S-adenosyl methionine (SAM), as well as S-adenosyl methionine into adenosyl-homocysteine (SAH) were both reduced in ewes on the MD diet. At day 6, the blastocysts were transferred to 203 normal eating ewes. Those that became pregnant gave birth to typical weight babies on both diets, yet the growth weight to weaning for the MD babies were higher, persisting at 22 months of age, resulting in heavier MD offspring. Male offspring of the MD diet experienced an increase immune response to a single intramuscular bolus of ovalbumin both at 1 year of age and in the weeks following the vaccination. The MD males were also proportionately fatter with less muscle mass than control males. MD males also showed the greatest response to an i.v glucose infusion, showing signs of glucose resistance independent of body fat. In terms of cardiovascular function, MD males showed 11 mm Hg higher blood pressure than the controls, as well as a higher systolic, diastolic, and mean arterial pressure response to angiotensin II infusions given in increments.

To test whether these results were linked to epigenetic modification, DNA was processed to look at 1,400 CpG sites using restriction landmark genome scanning (RLGS). Methylation sensitive restriction enzyme, NotI, was used to digest the unmethylated CpG sites, resulting in spots on the autoradiograph. Fifty seven loci were found to be altered in two or more MD animals, which is more than was expected if it were to be distributed randomly between the MD group and control group. Eighty eight percent of these loci were found to be unmethylated or hypomethylated compared to the control group. The other loci appeared to be hypermethylated. Again, a majority of these alterations occurred in males (Sinclair, et al., 2007). These studies continue to support the notion that the etiology of human disease can stem from epigenetic gene regulatory mechanisms that originate early in development from simple aspects of our daily nutrition. This knowledge can be utilized as a cautionary tale for intending mothers to start preventing the conception of unhealthy adults (Waterland and Jirtle, et al., 2003).

Toxicants

Bisphenol A

Bisphenol A (BPA) is an endocrine disruptor used in the manufacturing of polycarbonate plastics found in food and drink containers, baby bottles, and dental composites. Endocrine disruptors are chemicals that mimic endocrine hormones, interfering with the homeostasis of our various organ systems. The endocrine disruptor known as BPA has previously been linked to heavier body weight, increased risk of breast or prostate cancer, and abnormal reproductive function. BPA has been detected in 95% of human urine samples, attesting to the widespread exposure of BPA in the general public (Dolinoy, et al., 2007).

BPA is most known for its effect on genetic expression of homeobox gene Hoxa10, a gene that controls uterine organogenesis. Because BPA is an endocrine disrupting chemical, it has the ability to mimic the actions of these hormones in vitro, disrupting normal development. Hoxa10 gene expression alterations have previously been linked to human cancers, as well as endometriosis in females. BPA seems to work through an epigenetic mechanism to disrupt Hoxa10. In a study by Bromer et al., pregnant mice were treated with an intraperitoneal injection of BPA in sesame oil on days 9-16 of gestation, at a dose of 5mg/kg of maternal body weight. In comparison, mice in a control group were injected with just sesame oil. Offspring were then euthanized at two or six weeks after birth. Genomic DNA was treated with sodium bisulfite with unmethylated regions converted to uracil, while methylated regions remained unchanged. Via quantitative real time PCR, it was concluded the Hoxa10 expression increased by 25% after BPA exposure, as per the mRNA expression seen in comparison to controls. Immunohistochemistry, staining using antibodies specific for a target protein, showed consistent results, as Hoxa10 protein was seen throughout the uterus of female offspring. To determine if these changes were epigenetic in nature, bisulfite conversion, PCR amplicon cloning, and sequencing were done to determine DNA methylation levels. 100% of control mice showed methylation in the promoter region while only 38% of BPA-treated mice showed any sort of methylation. CpG islands in the intronic region showed methylation in 100% of control mice and only 57% of BPA-exposed mice. In summary, demethylation or hypomethylation was observed in all regions in all BPA-treated mice. However, no methylation changes were found in the pregnant mice that were treated with the intraperitoneal dose (Bromer, et al., 2010).

Hoxa10 gene is also known to be a weakly estrogenic compound, with the ability to bind to estrogen receptor α and β. With decreased methylation, Hoxa10 has an increased ability to bind to estrogen receptors (ER) . Therefore, the estrogen receptor element (ERE) of Hoxa10 becomes more occupied by estrogen receptor α and β, causing a heightened reactivity to estrogen levels. The health implications of this were examined by testing estradiol sensitivity in MCF-7 cells. MCF-7 cells are estrogen receptor expressing breast carcinoma cells, with a well characterized estrogen response. In this study, MCF-7 cells were transfected with either methylated or unmethylated ERE

containing Hoxa10-promoter sequence. The cells with unmethylated containing sequences had significantly increased activity with the addition of estradiol, while the methylated cells showed no change in activity (figure 6). These results can explain disease risks in later life due to increased estrogen responsiveness, perhaps explaining some reproduction system abnormalities or cancers of the reproductive system. In summary, these epigenetic changes that are spurred by uterine BPA exposure can be one of the suspects of health problems in adult life (Bromer, et al., 2010).

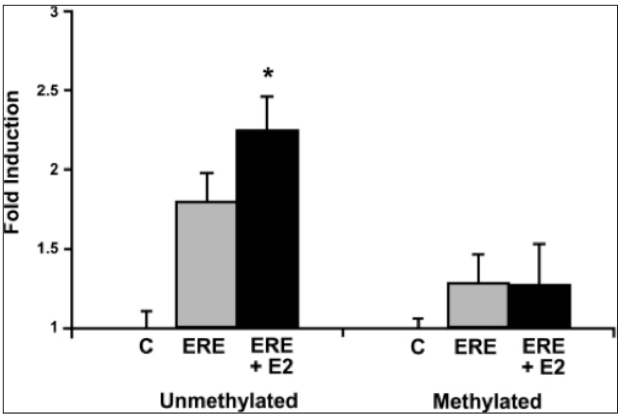

Figure 6 shows the reactivity of unmethylated Hoxa10-promoter to the addition of estradiol, while the methylated Hoxa10-promter shows a loss of response (Broner et al., 2010)

As mentioned previously, although most tissue-specific DNA methylation levels don't vary much across the mammalian genome, methylation is randomly determined at transposable element insertion sites. This can potentially affect the expression of neighboring genes, creating a site of metastable expression. As noted before, this site is particularly susceptible to environmental exposures early in development. The agouti mouse is a frequently studied case because it features the insertion of the intracisternal A particle, causing ectopic Avy transcription via a cryptic promoter at its proximal end, potentially leading to yellow fur, obesity, diabetes, and tumorigenesis (figure 6). Furthermore, methylation of the cytosines near the intracisternal A particle is inversely correlated to ectopic Avy expression and varies tremendously amongst isogenic individuals, causing a wide range of fur colors. BPA was hypothesized to demethylate this area, causing transcription and its phenotypic outcomes (Dolinoy, et al., 2007).

In this study, two groups of female a/a mice were prepared. The control received with one a phytoestrogen-free AIN-93G diet.. The experimental group received the diet with 50 mg of BPA/kg. Both diets were administered two weeks before mating with a Avy/a male until after gestation and lactation. There were 120 resulting offspring in the control group and 124 resulting offspring in the experimental group, with 60 and 73 Avy/a offspring, respectively. Maternal BPA exposure was shown to dramatically shift the coat color of genetically identical Avy/a offspring toward yellow, showing demethylation. 21% of offspring from the group exposed to BPA were labelled as yellow in comparison to 10% of the control group. To assess methylation levels, nine CpG sites in the cryptic promoter regions of the intracisternal A particle were measured using bisulfite treatment and sequencing. The BPA exposed offspring exhibited significantly lower levels in the nine CpG sites, with around 27% methylation compared to around 39% methylation in the offspring control group. After entering BPA dietary exposure, intracisternal A particle methylation, and offspring coat color into a mediational regression analysis model, it was determined that there was little relationship between BPA exposure and coat color directly, but rather methylation was the real mediator of effects. Methylation levels found in the tail corresponded to methylation levels found in the brain, kidney, and liver, revealing that these methylation patterns were established before germ layer differentiation in the embryo. BPA was also seen to affect other metastable epialleles, causing hypomethylation in multiple loci across the genome (Dolinoy, et al., 2007).

In an effort to test BPA levels in human subjects, 244 mothers and their 3-year-old children were recruited for a longitudinal study to measure BPA concentrations in maternal and child urine samples. Maternal urine samples were taken at week 16 and 26 of pregnancy and within 24 hours after delivery. Child urine samples were taken at ages 1, 2, and 3 years old. Behavior and executive functions of the children were also measured using the Behavior Assessment System for Children 2 (BASC-2), a 134-item test of a parent's assessment of a child's behavior in a home and public setting. Parents were also administered the Behavior Rating Inventory of Executive Function-Preschool (BRIEF-P), a 63 question, parental report test that analyzes the child's ability to modulate emotion, control behavioral responses (inhibition), plan for future events, set goals, grasp main ideas, transition from event to event, and hold information to complete a task. For both tests, higher scores indicated more severe impairment. It is intriguing that of the mothers who completed all the assessments, the majority were white, married, between 25 to 34 years old, wealthier, and more educated. Interestingly, these mothers had lower gestational BPA urine concentration than mothers who did not complete the assessment. Children from families of lower maternal education and income also had higher scores on BASC-2 and BRIEF-P.(Braun, et al., 2011)

Gestational urinary BPA concentrations were found to positively correlate with BASC-2 anxiety, hyperactivity, and depression scale scores at three years old, with the magnitude seen greater in females (almost double the association of the whole sample). Lack of emotional control and behavior inhibition suggest that BPA exposure may affect a neurobehavioral domain that is associated with behavioral regulation. Gestational urinary BPA concentrations were also positively correlated with emotional and inhibition scores on the BRIEF-P, with higher scores

attributed to girls again. In general, the effects of gestational BPA concentration were larger among girls than boys and had varying effects (boys had less hyperactivity). Neurobehavior and test scores were not predicted at all by childhood urinary BPA concentration. Only gestational BPA exposure can be implicated in altered neurobehavior, and specifically in females, as well.

The findings of these studies are consistent with previous animal studies that assess the effect of BPA exposure on neurodevelopment. BPA exposure might be responsible for the disruption of sexual differentiation in the brain, altering behavior in a "gender dependent manner," by disrupting endocrine or other neurotransmitter pathways. It is interesting to observe that all of the outcomes of BPA are traced to the exposures inside the womb, with no correlation at all to the postnatal environment (Braun, et al., 2011).

Remarkably, when the formerly mentioned agouti mice of the study done by Dolinoy et al. were supplemented with methyl donors or genistein in the experimental BPA diet, coat color distribution was observed as closer to what was seen in the control group (Dolinoy et al., 2007). Ten to thirteen percent of BPA- exposed -methyl/genistein supplemented offspring exhibited yellow fur color as opposed to the 21% seen in the non-supplemented BPA group, showing that maternal nutrition clearly modified the effects of BPA exposure on methylation patterns. Genistein at high levels had the same effect, even though it is not a methyl donor. Interventions as subtle as maternal nutrition is enough to counteract the effects of an estrogenic endocrine disruptor. This information implies that we are able to reduce disease susceptibility by making small changes in maternal nutrition, giving us the ability to affect generations to come (Dolinoy, et al., 2007).

Nicotine

The in utero effects of maternal smoking are infamous in their detrimental ways. Offspring exposed to smoking after birth do not experience the same adverse affects as those that are exposed as fetuses, hinting to a mechanism that is biologically mediated. It has previously been proven that rodents exposed to nicotine prenatally experienced a direct impact on brain development, reporting "abnormal dendritic morphology, and reduced synapse density in the cerebral cortex and nucleus accumbens" (Chatterton, et al., 2017). Nicotine exposure during gestation is also known to upregulate nicotinic acetylcholine receptors, causing cell death, altered cell size, and increased risk for behavioral impairment. A study done by Chatterton et al. examines the possible epigenetic modifications that are responsible for these developmental differences that have been found to persist even until adolescence. Fetuses aborted during the second trimester were studied and classified based on whether they were exposed to maternal smoking or not. In total, 24 fetuses were tested. The brains of the fetuses were removed to examine the dorsolateral prefrontal cortex, an area known to be involved in decision-making, memory, and neurodevelopment. It is also known to be compromised in function in different psychiatric conditions, including autism spectrum disorder. Methylation microarray analysis was performed to determine differentially methylated regions between the exposed and unexposed groups. Although differences did not remain significant after multiple testing correction (likely due to the small sample size), hypomethylation was found on two genes in the exposed fetuses; SDHAP3 and GNA15. The hypomethylation during second trimester of these two genes is indicative of developmental delays. This resulted in up-regulation of this gene as evident by the increase of mRNA in the samples, an effect mediated by maternal smoking (Chatterton et al., 2017).

Between early and late second trimester, many methylation changes are meant to occur to proceed with development. However, in fetuses exposed to maternal smoking, less change was observed. There was a delay in the upregulation of the SYCE3 gene, a gene that is responsible for synaptic initiation that results in meiotic arrest. As previously stated, SDHAP3 and GNA15 also displayed developmental delays. SDHAP3 is a subunit of the succinate dehydrogenase complex that functions in the electron transport chain. A mutation in this subunit can actually increase levels of oxidative stress. This same differentially methylated region was shown to be hypermethylated in patients with autism spectrum disorder and differentially methylated in the dorsolateral prefrontal cortex of patients with schizophrenia. GNA15 was also found to be differentially methylated in the prefrontal cortex of patients with autism spectrum disorder (Chatterton et al., 2017).

These results show a potential risk that smoking introduces to the fetus in the development of neural abnormalities. All in all, this study supports the concept that smoking exposure during gestation leads to changes in developmental patterns of DNA methylation and gene expression, causing reduced mature neuronal content via nicotine. Unfortunately, the damage can be everlasting (Chatterton et al., 2017).

Smoking during pregnancy has also been linked to obesity because of the shared presence of chemerin, an inflammatory adipokine that is responsible for adipocyte differentiation. It acts as a ligand for chemokine-like receptor 1 (CMLKR1), which is highly expressed in adipocytes. It is found to be elevated in both individuals who smoke and obese individuals.

Obesity has become a national epidemic with 35% of American adults and 20% of children obese, with a total of $200 million is spent each year in obesity-related healthcare costs. Finding the roots to this epidemic can help prevent and treat future cases. The hypothesis that the perinatal environment is responsible for the programming of adult disease is in question as it has been previously shown that fetal exposure to nicotine increases the risk of developing obesity and type II diabetes. The mechanism behind the correlation has yet to be confirmed,

as 15-18% of pregnant mothers continue to smoke throughout pregnancy and lactation (Reynolds et al., 2018).

In this study, two separate cohorts (2012-2013 and 2015-2016) of postpartum women were recruited from the University of Kentucky Chandler Hospital, Labor and Delivery Unit. Subjects were then identified as either smokers or non-smokers. All had full term pregnancies, singletons, and birthed male infants with circumcision performed less than 72 hours after birth. In the first cohort, foreskin tissue was then taken and dissected, snap frozen, and analyzed for chemerin mRNA expression. Of the 46 samples, 31 belonged to offspring of non- smokers with 15 belonging to offspring of smokers. Chemerin mRNA was analyzed in all samples while DNA methylation levels were analyzed in a subset of the samples (12 non-smokers, 7 smokers) (Reynolds et al., 2018).

In the second cohort, foreskin samples were collected from 24 newborns with 13 of them born to smoking mothers. Of these samples, only eight were able to be used due to cell contamination or lack of cell growth. These samples were dissected for the dermal/epidermal layer and incubated. The epidermis was separated from the dermal layer, and the dermal cells were processed and plated. RNA was then collected and isolated from the samples, and chemerin gene expression was analyzed from each sample using qPCR. Maternal and infant characteristics such as weight, height, and body mass index (BMI) were collected for both cohorts (Reynolds et al., 2018).

The results demonstrated that the weight and lengths of infants born to smokers were significantly reduced compared to those whose mothers did not smoke. The foreskin

Figure 7a and 7b compares chemerin mRNA expression and chemerin methylation in non-smoking exposed and smoking exposed infant foreskin samples. (Reynolds et al.,2018)

tissue of babies exposed in utero showed increased chemerin gene expression (figure 7a). Methylation percentages of chemerin CpG sites showed reduced levels of methylation. CpG site 3 of chemerin showed the most significant reduction of methylation with chemerin gene expression inversely correlated with methylation at site 3 (figure 7b).

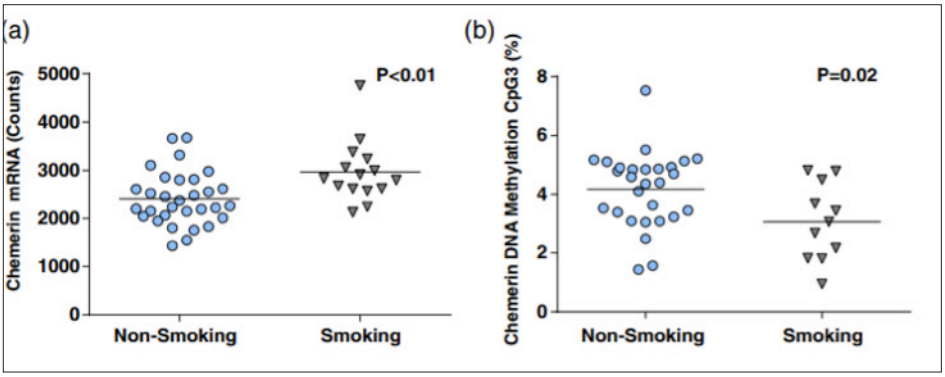

Figure 7a and 7b compares chemerin mRNA expression and chemerin methylation in non-smoking exposed and smoking exposed infant foreskin samples. (Reynolds et al.,2018)

The dermal fibroblasts that grew in cultures for the second cohort were then stimulated with an adipogenic cocktail. Babies of smokers produced cell plates that showed elevated chemerin gene expression, compared to the cells isolated from babies of non-smokers (figure 8). These results correspond with another study that demonstrates that although babies exposed to cigarette smoke in utero tend to be smaller, they have a greater rate of obesity as they get older, suggesting an altered developmental programming. This phenomenon known as, 'catch up growth,' puts babies at risk of developing cardiovascular disease, type II diabetes, or obesity later in life (Power, Jeffries, 2002).

This study involving the two cohorts supports the findings

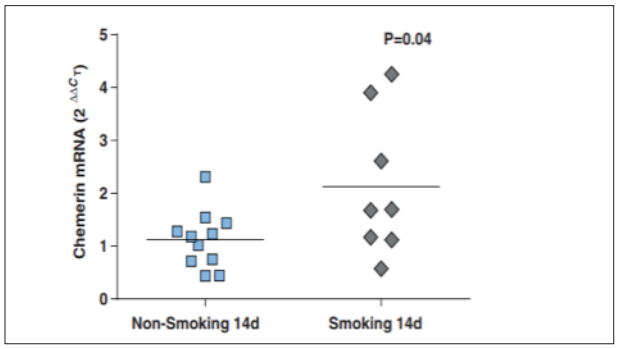

Figure 8, (Reynolds et al. 2017)

of Power and Jeffries et al., further linking cigarette smoking and obesity to methylation and chemerin gene expression. Of course, official causation cannot be completely determined without more research. The study has other limitations, as well: only testing male babies, testing dermal tissue and not adipose tissue, small sample size of the smoking group, and not taking into account second hand smoking are just to name a few. That being said, the overall conclusion is in agreement with other studies on this subject: Smoking alters chemerin gene expression in neonatal tissue of babies exposed to smoking, possibly causing obesity later in life. And perhaps the mediator in this change is epigenetic in nature (Reynolds, et al., 2018).

Trauma

Many people can be exposed to the same trauma, but only some will develop post-traumatic stress disorder (PTSD). So, it begs the question: Is there a biological basis or risk factor for the development of PTSD?

A cohort of 40 Holocaust survivors and their 31 offspring were studied in comparison to individuals of the same age that were not living in Europe in the time of the Holocaust.

Epigenetic changes in the FKBP5 allele, a glucocorticoid binding sequence at intron 7, were measured. Psychiatric analysis was also obtained from the subjects, showing that Holocaust survivors and their offspring suffered more from PTSD and anxiety and/or depression, respectively. After a whole blood examination, bisulfite treatment, and pyrosequencing were performed on the DNA isolates, both Holocaust survivors and their offspring showed differences in methylation on intron 7 of the allele at the bin3/site 6 region, in comparison to the control group. This highlights that Holocaust exposure in the parent was the sole predictor of methylation levels in the offspring. However, survivors showed 10% higher methylation, while their offspring showed significantly lower methylation (Yehuda, et al., 2016).

Offspring methylation levels were inversely related to their wake-up salivary cortisol levels, as well. This might be due to a "intergenerational biological accommodation" (Yehuda et al., 2016). A similar notion is seen with the 11β-HSD-2 allele, another moderator of glucocorticoid action. Increased activity of this allele in offspring was seen as a counteraction to the decreased levels of activity seen in mothers. In mothers, this decrease in activity was an accommodation due to high circulating glucocorticoids. However, in children, upregulation was needed to optimize glucocorticoid levels again. Similarly, because hypermethylation is occurring at the maternal FKBP5 allele to lessen the effects of circulating glucocorticoid, offspring exhibited demethylation to optimize glucocorticoid levels, as well. Although 10% less methylation seems relatively small, differential gene expression has previously been observed with methylation differences between 1%-2%. This lack of glucocorticoid sensitivity can lead to increased risk of psychopathology in the offspring generation, something that the offspring exhibited according to psychiatric evaluation (Yehuda et al., 2016).

A recent study that included Holocaust survivors, as well, discovered that parental PTSD is an important risk factor for the development of PTSD in offspring. Physically, children of survivors had lower 24 hour mean urinary cortisol excretion, an important symptom as lower cortisol levels are also linked to people who have suffered previous traumas. Previous trauma is another risk for the development of PTSD. If cortisol is associated with the risk of developing PTSD, prenatal influences must be investigated as the hypothalamic-pituitary-adrenal axis is programmed during early development. Maternal exposure to glucocorticoids while pregnant can lead to higher levels in the offspring and lower birth weight, a condition linked to various adult diseases including hypertension, insulin resistance, and depression, as well (Yehuda et al., 2005).

To test this correlation, 38 women from a larger cohort of 187 women directly exposed to the World Trade Center collapse on September 11, 2001 while pregnant, agreed to partake in a longitudinal study along with their children. At the ninth month checkup, mothers and infants were asked for their salivary samples at wake up and bedtime to assess cortisol levels. Probable maternal PTSD and severity were assessed with a PTSD checklist, and depression was assessed with the Beck Depression Index. Mothers with PTSD reported more depression than mothers without. Salivary cortisol levels in children of mothers with PTSD were significantly lower than those of mothers without PTSD, a trend that continued throughout the first year of infancy (Yehuda et al., 2005).

It is interesting to note that while the trimester of gestation did not affect maternal PTSD, the effect glucocorticoids had on the baby did differ slightly depending on the trimester of exposure. The babies born to mothers exposed in their third trimester showed even lower cortisol levels. That being said, the severity of PTSD symptoms in the mother correlated with cortisol levels in infants regardless of trimester (figure 9). The results highlight the critical windows of development that are available for reprogramming at each point of gestation, a principle that is important in most epigenetic modifications (Yehuda et al., 2005).

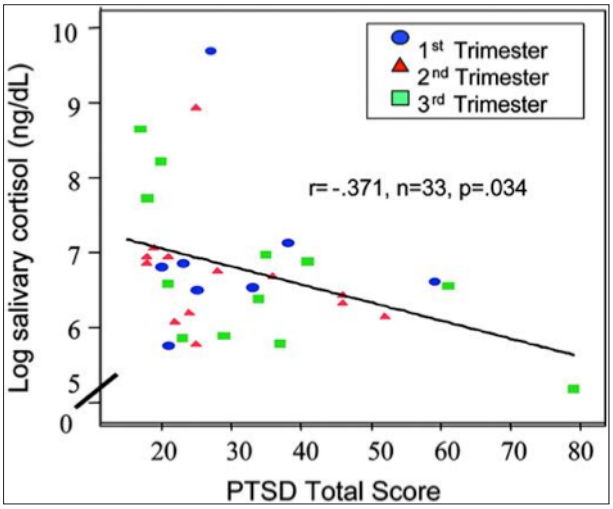

Figure 9, (Yehuda et al., 2005)

Many people are mistaken in assuming that children of Holocaust survivors or of parents with PTSD suffer mentally because of their postnatal upbringing by a traumatized and emotionally incapable parent. However, in Yehuda et al., 2016, the sole predictor for methylation levels in the offspring was parental exposure to trauma. According to the study, emotional abuse and childhood adversity actually did not show any significant correlation to methylation levels and glucocorticoid abnormality. This latter experiment is another testament to the effects the in utero environment can have on the future of the child, as opposed to postnatal "..vicarious traumatization of the offspring by the parents' communication of their trauma to the child or other consequences of parental symptoms (e.g. poor parenting)" (Yehuda, page 4). Babies in Yehuda et al., 2005 were only nine months old at the time of their endocrine testing, so glucocorticoid

programming in utero and/or underlying genetic susceptibility is more relevant then the postnatal environment. Yes, there is always the argument that postnatal care by mothers suffering from PTSD can show inconsistent behavior towards their offspring, affecting their glucocorticoid regulation. However, the stronger correlation to PTSD shown in offspring of the third trimester does implicate prenatal factors (Yehuda et al., 2005).

Stress

The hypothalamic-pituitary-adrenal axis controls growth, reproduction, metabolism, and behavior. It is also extremely active in a human's ability to 'defend' itself during stressful situations via the glucocorticoid receptor (GR). Transgenerational epigenetic modifications can affect the programming of the hypothalamic-pituitary-adrenal axis during early development, affecting glucocorticoid function, as stated previously. In a study that examined intimated partner violence during pregnancy, it was determined that the glucocorticoid promoter gene's methylation status can originate in the womb while a mother's remains unchanged, and sustain itself throughout adolescence. In this study, 25 mother and children pairs were evaluated to determine if gestational maternal adverse experience can cause methylation in offspring that persists for years after pregnancy. Children were between ten and nineteen years old at the time of the study (Radtke et al., 2011).

Mothers were given the Composite Abuse Scale (CAS) test three different times to evaluate intimate partner violence before, during, and after pregnancy (see Table 1). Blood was taken from the mother and child with which she was pregnant with at the time of abuse. The methylation status was evaluated at ten CpG sites found in a transcription factor binding site that are known to have their methylation statuses influenced by early life circumstances. Methylation was detected in 7 of the 10 CpG sites of the glucocorticoid promoter region, with methylation levels ranging from zero to 20%. There was significant correlation of methylation to maternal exposure to intimate partner violence that was experienced during pregnancy only, but no significant correlation was found between offspring methylation and intimate partner violence before or after pregnancy. There was also no correlation between maternal methylation and offspring methylation (figure10). This attests to the prenatal transgenerational influence that stress can have on the hypothalamic-pituitary-adrenal axis. This is consistent with previous studies that have shown that prenatal anxiety has caused sustained elevation of basal hypothalamic-pituitary-adrenal activity, causing behavioral and emotional problems that can continue throughout the lifetime of an individual. Methylation of the glucocorticoid promoter is the potential mechanism in which prenatal stress can influence psychological function. It is interesting that although this is transgenerational in nature, methylation was not inherited along the germ line, as there was no

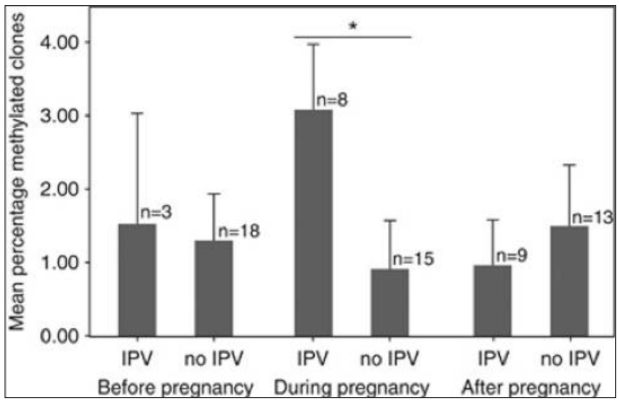

Figure 10

correlation between maternal methylation and offspring methylation (Radtke et al., 2011).

While direct stressors, like the aforementioned intimate partner violence on the mother, have been tested, indirect stress can also prove to be dangerous to a developing fetus. The perinatal environment is extremely impactful on the future of an offspring, with the malleable fetal brain susceptible to even the most subtle of experiences. Mychasiuk et al. studied the phenomenon known as "bystander stress" to demonstrate the effects it might have on the brain, behavior, and development of the epigenome. In this study, eight pregnant dams were separately caged with eight non-pregnant dams. On day 12 and 16 of gestation, the non-pregnant dams were placed on elevated Plexiglass platforms and exposed to bright light for thirty minutes, twice a day. In the control study, the cage mates were moved to another room twice a day for thirty minutes and returned unstressed. To examine data, researchers recorded ultrasonic vocalizations from the rats after the thirty minutes of stress were administered. After the birth of the 120 pups, the pups were also examined in various behavioral procedures to assess their brain development. Afterward their frontal cortex and hippocampus were examined for DNA methylation (Mychasiuk et al., 2011).

At baseline, all ultrasonic vocalizations were the same. However, after the stress procedure, the stressed dams had an increased number of low frequency calls while the bystander dams showed an increased number of high frequency calls. Previous research has demonstrated that low frequency calls are emitted during particular distressing situations, while high frequency calls are emitted in positive situations, such as in times of reward. The researchers suggest that it was the pregnant dam's attempt to soothe the distressed dam by emitting the high frequency call. The stressed dam had a loss of fur on female rats and were more aggressive toward their handlers than the pregnant dams. Once the 120 pups were born to the sixteen mothers, further analysis was done. Brain weight was not affected, but female pups exposed to "bystander stress" showed a decrease in body weight, confirming that the experience proved to be a stressful one (Mychasiuk et al., 2011).

Pups were then tested on their negative geotaxis to assess brain development. Negative geotaxis depends on "vestibular and proprioceptive detection, processing of the vestibular and proprioceptive inputs by the central nervous system, and motor competence to change orientation" (Mychasiuk et al., 2011). This is generally used to detect milestones in the sensorimotor development of rats. After being placed downward at a 40 degree angle, pups were tested on their ability to stay upward on the Plexiglass for the longest amount of time. Pups exposed to "bystander stress" spent less time facing upward on the Plexiglass platform with stressed females specifically receiving the lowest scores in the exercise. The pups were then tested in open field to assess their activity levels. The pups were placed in the center of a transparent Plexiglass box divided into 130 squares. Pups were scored based on how many novel squares they touched with their paw. The "bystander stressed" pups scored less than the control group, with females scores significantly lower than the males. This seems to show the hesitance and unwillingness to explore novel environments exhibited by these young pups. However, as they age, they will lose this inhibition, as seen in previous research (Mychasiuk et al., 2011).

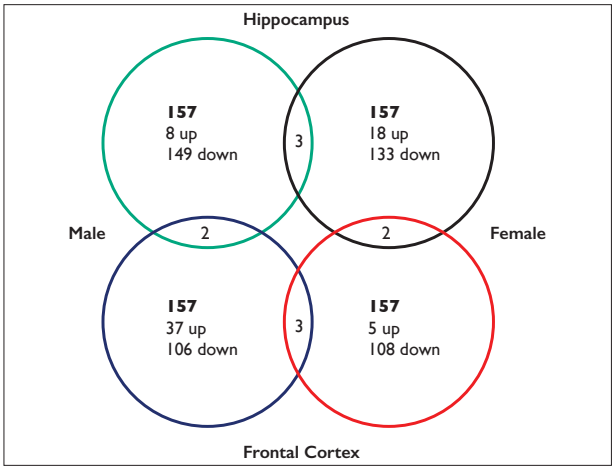

Figure 11 shows the differentially expressed genes found in stressed male and female pups.(Mychasiuk et al., 2011)

In terms of global DNA methylation, all animals in the "bystander stress" cages showed increase in methylation in the frontal cortex and hippocampus with a total of 558 genes differentially expressed between stressed male and female pups combined. There was minimal overlap of differentially expressed genes in brain regions between the sexes (figure 11). This suggests a preexisting difference in brain structure and genetic influence exhibited by males versus females, allowing them to respond differently to "bystander stress." This was clearly seen in their behavioral assessment after birth, as the females did significantly poorer than the males on both negative geotaxis, open field, and lower birth weight. That being said, while the genes did not overlap much, the biological processes that these genes control are very similar between the sexes, with most of genes controlling processes that are important for plasticity of the brain (i.e. cell communication, motion, and transport). Interestingly, a gene that was identical between the sexes in its abnormal expression was the SLC6A1 gene. This gene was downregulated in the frontal cortex. It encodes for a GABA transporter, removing GABA from the synaptic cleft. With its downregulation, GABA would be elevated in the frontal cortex, influencing processes like attention, response inhibition, and working memory. This study is groundbreaking in suggesting that indirect social stressors are just as harmful to the development of offspring as direct stressors, hinting to the intense sensitivity of the fetus. That being said, because blood was not drawn from the mother or "bystander" dam, the mechanism behind this stressed induced methylation cannot be understood completely and is in need of further experimentation (Mychasiuk et al., 2011).

It is vital to realize that while there is much evidence to support the concept that overactivation of the hypothalamic-pituitary-adrenal axis via heightened maternal glucocorticoids puts the infant at a great disadvantage that persists until adolescence, most experiments have been done with animals or small sample sizes. To properly test these results on humans, a cohort of 481 mother-infant pairs were taken from the Barwon Infant Study of Barwon, Australia. Maternal mental well-being was assessed using the Perceived Stress Scale (PSS) questionnaire, a well validated scale that assesses the biological impact of psychological distress. Maternal mental health was also tested using the Edinburgh Postnatal Depression Scale (EPDS) questionnaire at 28 weeks of pregnancy, a 10 item questionnaire used to assess symptoms of depression with a subscale of questions used to assess anxiety. A survey was also distributed to determine information on the physical and mental health of the mother, family medical history, lifestyle, demographic, education, maternal drinking, and other confounding factors. Many exposures were considered to eliminate narrow results that only reflected a specific measure of mental well-being (Table 1). Umbilical cord blood from infants were also collected from participants, as well as blood from the placenta before placental delivery, when possible. DNA was then extracted from the sample of blood and bisulfite converted using MethylEasy DNA conversion kits. Primers were used to amplify a 403 base pair region of the NR3C1 glucocorticoid promoter, spanning 47 CpG sites. After PCR amplification of the region, in vitro transcription and cleavage, DNA methylation was then quantified (Mansell et al., 2016).

The results revealed a strong correlation between the three tests. 65% of depressed mothers were also anxious, while 74% of anxious mothers were also depressed. Methylation levels of NR3C1 in three CpG units were shown to be higher in mothers with increased adverse maternal mental well-being; CpG 1.2, CpG 3.4.5, CpG 47. The aforementioned regions all correlated

to different mental well-being exposures. CpG 1.2 and CpG 3.4.5 were both correlated with perceived maternal distress. Maternal anxiety was correlated with CpG 47. Yet another region, CpG 12.13 was weakly correlated with maternal depression. The covariates like hypertension, preeclampsia, maternal age, antidepressant use, and smoking were correlated with hypo/hypermethylation at different CpG sites, independent of the three that were correlated to mental well-being. That being said, although this was the largest study of its kind performed on the association of methylation and mental-well being, after taking into account the multiple tests and numerous CpG islands affected by the three exposures and the covariates, none of the associations remained significant. This experiment essentially questions all previous experiments that were done with smaller sample sizes and lack of multiple testing. While there is consistency amongst the studies to suggest that it is more than just chance that links maternal depression, anxiety, and adverse well-being to methylation and offspring behavior abnormalities, this study highlights the caution needed in interpreting results and evaluating the studies without bias. The results do support previous findings, like the role of cortisol on fetal "programming," yet it also reveals how the proof is not yet strong enough to withstand a bigger sample size and multiple exposures. In conclusion, more studies, including longitudinal observations, are needed to fully link maternal mental well-being to methylation, as well as to determine the phenotypic implications (Mansell, et al., 2016).

Limitations

Although there has been much speculation and experimentation in the field of epigenetics, no study has yet to link the interplay between genes and epigenetics. When dealing with the cohorts in human studies, genetic dispositions might play a larger role in phenotypic outcome than is given credit. For example, in studies of trauma effect of Holocaust survivors, all subjects were of Eastern European Jewry (Yehuda et al., 2016). It is possible that the effects of methylation on the FKBP5 allele is specific to individuals within the gene pool of Ashkenazic Jewry, while other genetic population would not experience this specific outcome between genes and epigenetics. The fact that there are some Holocaust survivors that have PTSD and not others, also was not explored and hints to a genetic disposition, as well. Furthermore, many human studies relied on questionnaires that were geared toward the mothers or children that were being studied. It is hard to believe that every subject answered with only factual information as opposed to biased and emotional responses. These studies already create a bias from the start, as all subjects consented to the study, thereby aware of what the study was about. Perhaps they agreed because of their feelings of relevance, removing randomness from the population. Another limitation as discussed in Mansell et al. concerns the sample sizes and multiple testing of the experiments. Most of the sample sizes, especially in the human experiments, are not big enough to be relied on completely. Even many of the animal experiments involved less than 100 samples, something that definitely takes away from the credibility of the results. Furthermore, as Mansell et al. clearly stated, multiple testing procedures usually eliminate any significant correlation found. While there is definitely integral information for epidemiology within the field of epigenetics, future experiments and reports should focus on expanding the sample size and designing experiments to survive multiple testing (Mansell et al., 2016).

Conclusion

Epigenetics refers to covalent bond changes that happen above the level of DNA that affects gene expression. Many researchers have speculated about a link between the prenatal environment and adult health. This link is thought to exist because of epigenetic alterations that happen to the fetus because of different environmental exposures. Epigenetic programming takes place during early development in the fetus, thereby presenting a vulnerable and malleable setting for modification.

The purpose of this paper was to investigate the possible link between the in utero environment and adult health, along with the biological mechanism behind it. While there were many limitations to the studies as previously mentioned, there is overwhelming proof that environmental exposures like nutrition, vitamin B, smoking, BPA, stress, and trauma have negative effects on the health and quality of life of offspring. Studies have linked prenatal exposures to obesity, hypertension, coronary artery disease, cancers, and mental illness. According to the aforementioned studies, the events a mother experiences and the decisions that she makes during gestation can seriously deter the path of health for her future adult child. More research and samples are definitely needed to further back this hypothesis, but there is unquestionably enough evidence to support the correlation. It is also important to note that it is possible to avoid some of the detrimental effects of these exposures. As seen in Dolinoy et al., a simple methyl supplement was enough to modify the effects of BPA on an exposed mouse (Dolinoy et al., 2007).

There is no shortage of adult disease victims in America. The Heart Disease and Stroke Statistics of the American Heart Association stated that as of 2019, 46% of Americans suffer from hypertension. The National Institute of Mental Illness stated that 1 in 6 Americans suffer from mental illness. By educating expectant mothers, maybe we can prevent the development of adult disease in at least a percentage of patients. Furthermore, as Powledge mentioned in her article, epigenetics is not permanent like our DNA. More research in this area can help us learn how to rid ourselves of the modifications, or use these modifications to rid ourselves of other undesirable genetic information (Powledge, 2009).

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