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Keywords
epigenetics, genetics, glucorticoid receptor

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Introduction

Since the discovery of its structure and function, DNA has been named the blueprint for every living organism. But since the discovery of epigenetics, that blueprint began to look more like a rough idea than a finished thought. There are many ways to describe epigenetics, but the simplest description is environment affecting DNA. The genetic code of an organism governs everything within the organism: protein production, hormones, organ synthesis, and various physical traits. Epigenetics works by acting as a liaison between the genetic code and the output. The simplest epigenetic modifier is a DNA methylation; for example, a particular nucleotide of DNA is methylated, turning cytosine into a methyl-cytosine. Modifying methylation of various nucleotides allows for the second epigenetic modifier to alter the DNA transcription histone modification. While the methylation of the nucleotide is more of an on or off switch, the histone modification acts like a dial, varying the DNA translation by winding DNA more tightly or loosely and making it more or less accessible to translational proteins. DNA methylation and histone modification can be altered by environmental factors of an organism, abundant food, scarce food, stress, exposure to drugs, toxins, hormone replacement chemicals and endocrine disrupting chemicals (EDCs). These factors play a major role in a natural ecosystem, but far different rules come into effect as the focus shifts to human epigenetics. Humans choose the food they eat, the amount of exercise they complete, and the drugs they take. These choices have been shown to alter gene expression in statistically significant ways, and not only are the individuals that originally made the choice affected, but their offspring, and even their offspring's offspring, are affected (Curley, Mashoodh, &

Champagne, 2011).

Historically, epigenetics is limited to physiological factors such as weight, certain types of cancer, birth defects, and fertility problems. However, the epigenetic alteration being studied in this paper is psychological, and deals with escalated stress responses in certain individuals. This is a tough hypothesis to test experimental conditions, since most of the environmental factors that could have a far reaching genetic change on an individual's stress response also have short term factors that could be the only cause of the stressed status. In sum, epigenetic factors can have an effect on the genome of an individual and these factors are inheritable in the germ line. While most of these factors are physiological, there are some that, in turn, affect the psychology of individuals and can leave lasting effects in adulthood and into the next generation.

Crash Course of Epigenetics

On a chemical level, a DNA nucleotide is methylated by some environmental factor, possibly domestic violence, or more likely a poor diet and lack of exercise. The methylation changes the way that nucleotides are read by the ribosomes of the cell in one of two ways. The first modification is the methylation of nucleotides, preventing transcriptional proteins from binding to the nucleotide in the first place. The second modification pathway is the methylation of the nucleotide allowing for methyl-CpG-binding domain proteins. These proteins allow for two other types of proteins to come to the location and further modify DNA. The two proteins are histone de-acetylases and various chromatin remodeling proteins. The chromatin remodeling and histone de-acetylases change the transcription of the DNA by winding the DNA more loosely or tightly and

helping or preventing translational proteins to access the DNA. These results can be seen in studies that observed two distinct lineages of obese and fit rats under test conditions, isolated DNA methylation sites, and correlated them with the raw data. The groups of rats were given standardized high fat diets (HFD) or low fat diets (LFD) for a period of 32 weeks. The groups consisted of HFD and LFD groups in each rat lineage for a total of four groups. After the test period, the rats' epigenetic profiles for a particular gene Mc4R were extracted and interpreted. The LFD groups for both rat lineages yielded no results on methylation of the Mc4R gene, but in the HFD groups of each lineage the obese rats had no differences in the methylation of their genes while the fit rats had a statistically significant lower methylation of the Mc4R gene. Counterintuitively, the obese rat colony had no improvements in either their weight or methylation of the gene regardless of a better diet for a long period of time (Widiker, Karst, Wagener, & Brockmann, 2010). The Mc4R gene is a melanocortin receptor responsible for feeding behavior, sexual behavior, and metabolism. Demethylation of the locus responsible for encoding the Mc4R gene would not cause the fit rats to become obese in the 32 weeks of the experiment. Instead, the diet would cause the obesity before the gene could. However, demethylation of the gene is a harder problem to fix than diet and exercise. In this regard, epigenetics is a lasting impression of choices made and can make reversing the effects of said choices much more difficult than it should be, just like the obese rats making no improvements regardless of their diets (Widiker et al., 2010).

Heritability is one of the most important topics in epigenetics. It can be a blessing for some and a curse for others depending on the lifestyles of their descendants. In a review by

Crews, effects of many hormone disrupting chemicals (HDCs) were explored as well as the lineages of those affected. Crews (2006, S5) states "Treatment with diethylstilbestrol (DES) during pregnancy results in vaginal adenocarcinoma in human and mice female offspring. Female offspring of mice exposed to DES during pregnancy, when mated to control males, produce a second generation of females who, although not exposed to DES themselves, express this same rare genital tract cancer." When chemicals are discussed in accordance with biology, most of the effects are deemed long-term exposure or acute. The results of this experiment are counter intuitive, since the mothers who were directly exposed to the chemical while pregnant have no alterations at all, but their offspring as well as the F2 generation have the genital tract cancer.

A separate study observing the male offspring of female rats exposed to DES found that the F1 generation of male rats had fertility issues as well as F2, F3, F4, and even F5. (Crews & McLaughlan, 2006). Because of the high penetrance in several generations after the exposure to the EDCs, an epigenetic correlation was deemed to be the culprit. A separate study by Li, inspired by the first study involving vaginal tract cancer, sought out to determine if mutations or epigenetic factors were responsible. Li and colleagues found that the c-*fos* gene in the mice was completely unmethylated and the mRNA levels were 1.4 to 1.9 times higher than normal. The c-*fos* gene is associated with progression of cancer when overactive due to over expression of cyclin D1, A and E (promoters for mitosis) in osteoblasts and chondrocytes. Without a methyl group to inhibit the gene, it was fully expressed resulting in cancer (Li et al., 2003). The mice and humans were diagnosed with cancer in the vaginal regions because DES was originally a medication

designed for sexually transmitted infections and would concentrate around the genitalia.

Stress

Stress correlations can come from many sources, but mainly emotional trauma. One such study of stress was performed by Radtke and colleagues (2011) involving intimate partner violence (IPV) of mothers before, during, and after gestation. Twenty-nine mothers whom experienced IPV before, during or after pregnancy volunteered themselves and their offspring for this study. Maternal exposure to IPV, degree and frequency of exposure, offspring gender, child age, mode of birth, maternal age at birth, childbirth weight, and mother's country of origin were recorded. The gene under observation was the glucocorticoid receptor (GR) gene. This gene regulates the GR responsible for stress response. All of the mothers in the study had an increased methylation of the GR gene, which in this case was acting as a suppressor for the gene. The offspring had a statistically significant increase in methylation of the GR gene only if their mothers were exposed to IPV during pregnancy. Exposure to IPV before or after pregnancy had no effects on the offspring's methylation of the GR gene (Radtke et al., 2011).

Another study involving the GR gene was done using rats and their devotion to their offspring. Mother rats in captivity that recently gave birth were observed in their reactions with their offspring shortly after birth. Licking and grooming (LG) behavior was observed and designated low, adequate, or high LG behavior, measured by the time mothers spent with their offspring over their first week of life. Increased maternal LG behavior is responsible for thyroid hormone-dependent increase in serotonin (5-HT), this increase of 5-HT is responsible for

demethylation of a promoter gene and as a result histone modification and amplification of nerve growth factor-inducible protein A (NGFI-A). NGFI-A binds to the exon 1_7 GR binding site and reduces the transcription of the GR gene. The results of this study were influenced by the environment, with rats receiving inadequate LG behavior displaying a reduced GR expression, and with rats receiving inadequate LG behavior but given to foster mothers with adequate care displaying normal GR expression. The GR expression of the rats would set within 10 days of birth, but de-acetylase inhibitors could reverse the epigenetic effects in the rats receiving inadequate LG behavior and the offspring would have normal GR expression (Weaver et al., 2004).

GR expression is an important element of organismal stress response. The GR is actually the cell receptor that initiates the fight or flight response throughout the body. The hypothalamic-pituitary-adrenal axis (HPA) is the originator of the stress response within the body. The HPA is the mediator that determines whether or not to initiate the fight or flight response with the hypothalamus at the top of the chain linking brain activity to the endocrine system. The next step is the anterior pituitary gland, which secretes releasing hormones and releasing factors that give instructions to the other major endocrine glands throughout the body. The final step in the HPA axis is the adrenal gland(s) located on top of the kidneys. These two glands are responsible for sending glucocorticoids and mineralocorticoids through the bloodstream, preparing the body to fight or run in the midst of an imminent threat. Abnormal responses to stressful situations and certain mental disorders are normally attributed to a malfunctioning or overactive HPA axis. Sometimes the culprit is not the message itself, but regulation of

the message by the receptors. Kolber and colleagues (2008) put two groups of mice in standardized stressful situations and evaluated their responses. The stress situations were the open field test, the elevated plus maze (EPM), the elevated zero maze (EOM), and light: dark (L:D) preference. One group of rats was genetically altered to have an increased GR expression while the other rats were altered to have a decreased GR expression. The mice with two extra copies of the GR gene showed a twofold reduced expression of stress hormone and stress response while the mice with reduced GR expression showed significantly elevated stress hormones and stress responses under test conditions. (Kolber, Wieczorek, & Muglia, 2008).

A study by Ouellet-Morin and colleagues (2013) established a link between adversity and methylation of the serotonin transporter gene, by recruiting participants from a parent study of 1,116 sets of monozygotic twins from England and Wales between the ages of 5 and 10. Only 28 sets of twins were applicable for the sub-study; criteria for the sub-study were that at least one twin was bullied occasionally, the bullying was reported by parents and the bullying was both psychological and physical. The sets of twins had no large variation of problem solving, IQ, or family living conditions. Saliva samples were taken and DNA profiles were developed and cortisol response data was generated. The twin that was bullied in each study showed a statistically significant increase of methylation of the serotonin transport gene. The bullied twin also displayed a significant decrease in cortisol response. This decreased cortisol response was deemed to be a down-regulation of HPA axis activity after overexposure to cortisol and this is known as the attenuation hypothesis (Ouellet-Morin et al., 2013).

A study by Booij and colleagues correlate many factors

present in the aforementioned epigenetic alterations. The serotonin neurotransmitter has been well researched for over 60 years, but recent studies are putting the pieces together forming a larger picture. Since 1966 low serotonin was known to cause depression and in 1976 was determined to be a factor of suicide. In 2014, serotonin was listed as a vulnerability factor for stress related disorders. Development of neurons displaying serotonin activity appear on days 10 to 12 in rat gestational periods and the serotonin is required for neurogenesis, dendritic maturation, axon connectivity, synaptic plasticity and apoptosis. Serotonin is critical during early to adolescent growth and development and if not enough is supplied, brain development may be disrupted. The serotonin transporter (SERT) gene is responsible for transporting serotonin from the synaptic cleft to the presynaptic neuron. Absence or inactivity of this gene causes serotonin deficiencies and several neurological disorders ranging from depression, increased aggression, psychopathy, alcoholism and obsessive compulsive disorder. Methylation of the SERT gene acts as an inhibitor for the gene, preventing transcription and serotonin transport (Booij et al., 2015).

Glucorticoid Receptors and Serotonin

The stress response from all three studies seems arise from adversity or early life problems and neglect, affecting serotonin levels and GR receptors simultaneously. These problems not only imprint on a newly formed life born into unfavorable conditions, but also serotonergic deficiencies are set in place and are hard to remove past a certain point. If after 10 days the rats received inadequate LG behavior, the GR receptors were expressed less as a result of the serotonin deficiency, leading the rat to display anxious and erratic behavior, all

because of a lack of grooming behavior for a short time after birth.

Transitioning to human developments, the bullied twins in Ouellet-Morin's (2013) study all had a statistically significant methylation of the SERT gene responsible for serotonin transport. A direct connection was made between the studies involving the SERT and GR genes. The pathway consists of: Early life hardships → SERT methylation → 5-HT serotonin deficiency → 5-HT not binding to NGFI-A promoter → no binding to the $exon_7$ GR promoter → lack of GR gene expression → increased stress response. The study performed by Booij and colleagues (2015) found a correlation between 5-HT serotonin and several mood disorders including psychopathy and depression, with Booij stating, "this [serotonin deficiency] in turn may affect brain development, including the hippocampus, a region with dense serotonergic innervations and important in stress-regulation." (2015, p. 12). The correlation found was traced back specifically to stress response. The results of an elevated stress response are numerous Not only are stress prone individuals much faster to reach a stressed state, but the stress response is much stronger, eliciting feelings of fear and clouding judgment. Over many years, mental and physical health problems arise as a result.

Epigenetics: Environment or Genes?

A mutation in genetics is defined as a change of the nucleotide sequence of an organism. Most commonly, the mutation does absolutely nothing to the organism in question; 98% of DNA is in non-coding regions meaning that 98% of the already rare mutations that happen in DNA proofreading are not observable by physiological changes. Mutations are also

heritable, passing in the germ line from one organism to the offspring. Mutations sound very close to epigenetics, and most of the refutations for epigenetics would be that the observable change in physiology is a mutation rather than an epigenetic change. With epigenetics, there are also environmental factors at play altering genetic expression, and it becomes difficult to draw the line between the environment dictating behavior and influencing it on a genetic level. A child born into an abusive household might have an elevated stress response only because a stressful environment is all he knows, but he may also have an elevated stress response because the abuse in early life altered his genetic expression and made him more prone to GR gene under-expression. Correlations in epigenetic tags are relatively easy to establish regarding metabolic processes, and even certain types of cancer due to the nature of these issues. However, when epigenetics is applied to psychology the correlation is not as easy to distinguish. Regardless of an individual's upbringing and increased tendency of emotional outbreaks, behavior is often not instinctual, but rather a mental process involving choices; and choices are much too complex to correlate genetic factors with.

Conclusion

Epigenetic factors certainly play a role in shaping the way human stress response adapts to everyday situations. Originally developed as a self-preservation tool to increase survivability, this response has adapted to the modern world, activating when papers or projects are due, and helping to save lives when emergencies strike. However, this stress response has also evolved to become hyperactive if the environment is not stable during early years of development as a coping mechanism to help endure hard times throughout life in an inhospitable

environment. These epigenetic changes to stress response happen primarily as a reaction to emotional trauma; these traumatic events set in motion a cascade of genetic and physiological responses. Of these many responses, the most common is reduction in gene expression for several protein binding factors and congruently a gene that regulates the glucorticoid receptor. When the genes for this receptor are below normal levels, the receptors are hyperactive and cause the elevated stress response. These increases in stress response are heritable to a certain degree, and can have negative effects on offspring despite a low stress environment. The ramifications of this increased stress response range from a higher amount of daily stress, to psychopathy, to severe depression. Finding the root cause of psychopathy and severe depression could have a plethora of future benefits. With modern medicine, the symptoms of the disease instead of the cause of the disease are treated, resulting in potentially inadequate treatment and in special cases, exacerbation of the disease. Using epigenetic studies, gene therapy could be a viable option to reverse and maintain these epigenetic factors, and some of these are already in use today. For example: de-acetylase inhibitors were used in the study involving LG behavior to reverse the effects of inadequate LG by mother rats and return the pups to a baseline GR expression. With further research, de-acetylase inhibitors and treatments to alter methyl groups on nucleotides could be used to treat and even cure many diseases that currently have no successful remedies.

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