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Effects of Stress on Human Genome
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Genetics of the Stress Response and Stress-Induced Disease

Although there is much disagreement over how best to define "stress," the condition is often described as a state of disturbed homeostasis that induces a variety of physical and mental adaptive reactions. These physiological changes are generalized as the stress response, which aims to restore the body to initial homeostatic levels after being aggravated by a threatening stimulus. Sometimes, if an organism is continually subjected to a stressor, the original condition of homeostasis cannot be achieved. After successful recovery from the stress response, the individual is said to equilibrate at an allostatic state—a new condition of "normal" that is different from the initial state of homeostasis.

Stressors, factors capable of triggering the body's stress response, can be physical or psychosocial. Internal, physiological characteristics of the stress response include increased heart rate and elevated levels of cortisol, epinephrine and norepinephrine in the blood. An individual engaged in a stress response may also exhibit external symptoms, such as muscular trembling, increased perspiration and clenching of the jaw. Successful coping with stress is characterized by the individual's ability to voluntarily or involuntarily regulate the magnitude of the stress response, and the ability to terminate the stress response when the stressor is removed or homeostasis is re-achieved.

Research suggests that the expression of neuropeptide Y (NPY) may determine an individual's predisposition to successful stress coping. NPY belongs to a class of compounds that inhibits anxiety. The release of this anxiolytic peptide from the limbic system of the brain is triggered by stress-inducing stimuli. Studies show that low expression of NPY is predictive of higher "emotion-induced" activation of the amygdala and decreased resiliency of opioid neurotransmission in brain. Stress, both physical and emotional, activates release of internal opioids—compounds that bind to opium receptors in the brain and decrease the perception of pain. Geneticists have discovered that a single nucleotide polymorphism, SNP rs16147, in the promoter region of the NPY gene alters NPY expression, both in vivo and in vitro. The presence of this SNP determines that extent to which the individual is resilient to stressors. It has been found that a decreased expression of NPY is correlated with an individual's heightened emotional response to stress, and decreased ability to quickly recover from an activated pain or stress response.

The activation of the SNS and hypothalamic-pituitary-adrenocortical (HPA) axis are two mechanisms largely responsible for restoring internal homeostatic conditions once the threat or stressor has been eliminated. In response to a stressful event, SNS is activated at the hypothalamus, which triggers the release of the hormones noradrenaline and adrenaline from the adrenal medulla. When the hypothalamus is stimulated, the neuropeptides corticotropin-releasing-hormone (CRH) and vasopressin (AVP) are released into the bloodstream. It is the SNS activity activates at the hypothalamus that regulates the HPA axis. The release of CRH and vasopressin signal the release of corticotropin (ACTH) from the anterior pituitary. ACTH then causes the adrenal cortex to synthesize cortisol, a glucocorticoid hormone that is necessary for the body's adaptation to acute stress conditions. Cortisol is essential for the restoration of homeostasis after a stressful experience and is extremely beneficial when it is properly regulated. When cortisol levels are consistently elevated, however, the hormone can become pathogenic, putting the individual at risk for a multitude of stress-induced health complications.

Malfunction, typically overstimulation, of the HPA axis is likely the cause of various stress-related disorders. The glucocorticoid receptor gene, NR3C1, is largely responsible for the regulation of the HPA axis. The NR3C1 gene is activated in response to elevated corticosteroid levels, and its protein, the glucocorticoid receptor, functions in terminating the stress response, and thus facilitates recovery from the stressful event. Although it may be difficult to directly study the effects of stress on the human genome, much can be learned through the analysis of stress-related disorders. Most individuals affected by chronic stress-induced diseases share genetic mutations specific to their condition. By analyzing the genome of such individuals, researchers can infer how the mutated genes moderate the individual's inappropriate stress response. Stress-related disorders fall within two main categories: cardiovascular disorders and psychiatric illnesses. Hypertension and coronary artery disease are examples of cardiovascular disorders, while bipolar and unipolar depression exemplify psychiatric complications. The following section of this paper discusses the genetic basis of these diseases and explores how these conditions result from irregular stress response.

Hypertension is the condition of chronically elevated arteriole blood pressure, persistently at or above 140/90 mmHg. High blood pressure is a risk factor for stroke, heart attack and heart failure, and, if left untreated, is often indicative of a shortened lifespan. Genetic predisposition for hypertension is likely caused by mutation in the genes of the renin-angiotensin-aldosterone system (RAAS): renin (REN), angiotensinogen (AGT) and aldosterone synthase (CYP11B2). The RAAS pathway is the main mechanism through which the body regulates blood pressure. The REN gene, located at chromosome position 1q32, functions to raise systemic blood pressure. The renin protein acts in the first step of the RAAS pathway to cleave angiotensinogen to form angiotensin I. Angiotensin I is then cleaved to form angiotensin

II. The AGT gene, located at chromosome position 1q42-q43, encodes the pre-angiotensinogen protein, which is highly expressed in the expressed in the liver. The renin enzyme cleaves the pre-angiotensinogen protein when blood pressure is low. Vasoconstriction is triggered and effectively elevates blood pressure back to homeostatic levels. The CYP11B2 gene on chromosome 8q21-22 encodes a protein that is a member of the P450 superfamily of enzymes. These enzymes catalyze the synthesis of steroid hormones such as aldosterone. Aldosterone increases reabsorption of water and ions in the distal tubules and collecting ducts of the kidney nephron. This process also increases blood pressure by increasing blood plasma volume. Genetic mutations increasing the expression of the REN, AGT and CYP11B2 genes may contribute to the condition of hypertension.

Several gene x gene interactions have also been determined to play a role in the causation of hypertension. Studies suggest the interaction between the endothelin 1 (EDN1) and serotonin receptor 2a (5HTR2A) genes is one such example. Both the END1 gene on chromosome 6p24.1 and the 5HTR2A gene on chromosome 13q14-q21 and encodes proteins that induce vasoconstriction. The ACE aldosterone synthase (CYP11B2) gene also interacts with the alpha adductin (ADD1) gene to increase reabsorption of water into the bloodstream to elevate blood pressure.

In addition to hypertension, coronary artery disease is another stress-induced cardiovascular condition. Although many studies have been conducted to determine the genetic basis of coronary artery disease, the results are difficult to ascertain with certainty, as both myocardial infarction and arteriosclerosis/stenosis are generalized as coronary artery disease. Significant associations have been made between mutations in genes involved in the innate immune response and inflammation process, and the elevated risk for myocardial infarction. Myocardial infarction, colloquially termed heart attack, is a condition in which the heart muscle is deprived of blood flow. Most heart attacks are physiologically caused by the occlusion of one of the coronary arteries vascularizing the muscle. Symptoms of heart attack include shortness of breath, chest and left arm pain, abnormal heart palpitations and feelings of weakness, fatigue and anxiety. If the condition is not treated immediately, myocardial infarctions can be fatal.

Although there are many lifestyle risk factors for heart attack, such as old age, smoking, excessive alcohol consumption, lack of physical activity and high-cholesterol diet, there is also strong evidence of a genetic basis for this disease. The toll-like receptor 4 (TLR4) gene involved in the body's innate immune response demonstrates significant associations with acute coronary events and infarctions. This gene is located at chromosome position 9q32-q33. Most commonly, this gene functions in cooperation with the LY96 and CD14 genes to mediate the human body's immune response to invading bacterial lipopolysaccharides. Almost all gene ontology terms listed for TLR4 in the Uniprot database are associated with the immune response, including the

positive regulation of platelet activation. Although most coronary artery occlusions are caused by an accumulation of white blood cells, cholesterol and fat, it is conceivable that excess platelet build-up may also contribute to the blockage. It is possible that an overproduction of blood platelets in response to an upregulation of the TLR4 gene may aid in inducing myocardial infarction. Research suggests that upregulation of the TLR4 gene in combination with chronically high stress levels serve as risk factors for heart attacks.

Evidence suggests that malfunctions in the leukotriene A4 hydrolase (LTA4H) gene may also serve as a risk factor for myocardial infarctions. The LTA4H gene is located on the q arm of chromosome 12, position 22 and plays a role in the inflammatory response. This gene is associated with the leukotriene biosynthetic process and proteolysis Uniprot gene ontology terms. The former biological process may be more closely connected to increased risk of heart attacks: the leukotriene biosynthetic process is defined by a series of chemical reactions and pathways through which leukotrienes (compounds mediating the inflammatory response) are formed from polyunsaturated fatty acids. The build-up of saturated fatty acids—which often exist in the body in a solid state—is more commonly considered a risk factor for heart attack. It is plausible, however, that excess polyunsaturated fats may also contribute to the condition, thus indirectly linking the LTA4H gene to the development of myocardial infarction.

Bipolar depression disorder is a psychological condition that is thought to develop as a result of high levels of chronic stress. Bipolar disorder, also known as manic depression, is characterized by unusual mood shifts and great variation in energy and activity levels throughout the day. This condition greatly detracts from the quality of life of affected individuals, and can damage interpersonal relationships, reduce work performance and may result in suicide. This condition is highly hereditary, at an estimate between 80-90%. Many studies identify a strong association between malfunctions in the monoaminoxidase A (MAOA) and catechol-o-methyl-transferase (COMT) genes and the development of bipolar disorder. The MAOA gene is located at chromosome position 5q31.3 and its encoded enzyme catalyzes the breakdown of amines such as dopamine, norepinephrine and serotonin. Dopamine is the neurotransmitter responsible for reward-motivated behavior and plays a big role in one's ability to maintain focus on a task. Norepinepherine stimulates many biological processes, and low levels of this neurotransmitter create a low-energy, decreased focus state. Serotonin is necessary for the maintenance of stable mood balance and is largely responsible for feelings of happiness. It is evident that a mutation in the MAOA gene could decrease the individual's capacity to regulate the activity of these essential neurotransmitters, many of which play a role in mood regulation. Thus, an individual possessing the mutated MAOA gene is at elevated risk for developing bipolar disorder.

The COMT gene is located at chromosome position 22q11.21 and its protein is responsible for transferring methyl groups to catecholamines, such as the neurotransmitters

dopamine, epinephrine and norepinephrine. Epinephrine and norepinephrine are hormones essential to activating the "fight or flight" response of the SNS. With a mutated COMT gene producing damaged proteins, there would be compromised methylation of such neurotransmitters. Gene methylation is thought to play a crucial role in the down-regulation and inactivation of gene expression. Without regulated methylation of epinephrine and norepinephrine, these hormones will be activated for a prolonged period of time, resulting in the constant stimulation of the flight or flight response. It is conceivable that continual stimulation of the SNS could be damaging to the organism, as the fight or flight response is designed to help the body cope with threatening situations, and signals activation of the HPA axis to regulate the stress response. Irregular activity of the SNS and HPA axis can result in abnormal cortisol levels in the body, which may intensify symptoms of the bipolar condition.

The COMT gene also plays a role in the metabolism of drugs used to treat hypertension. An individual's rate of drug metabolism determines the duration and intensity of the drug's response in the body. Malfunction in the COMT gene may also be responsible for decreased effectiveness of drugs intended to reduce elevated blood pressure; therefore, mutations in the COMT gene may have negative effects on two stress-induced diseases, hypertension as well as bipolar disorder.

Unipolar depression is another neurological disease likely caused by chronically high stress levels. Symptoms of this condition include persistent feelings of unhappiness and defeat, low-self esteem and loss of interest in or no motivation to partake in previously enjoyable activities. Incidence of unipolar depression is severe and widespread: the CDC reports that 1 in 10 US adults are depressed and approximately 15% of clinically depressed patients commit suicide. Estimates for the heritability of unipolar depression fall between 33 and 42%. There is strong evidence that unipolar depression is caused in part by a stress-induced mutation in the tryptophan hydroxilase 2 (TPH2) gene. Studies show that this gene, located at chromosome position 12q21.1, is stimulated by glucocorticoids and is heavily expressed in the human brainstem. The TPH2 gene is responsible for the rate-limiting step in serotonin synthesis, a neurotransmitter closely linked to feelings of happiness.

In a study published in a 2003 edition of *Science* magazine, researchers investigated whether some individuals are genetically predisposed to developing depression after repeated stressful experiences. The goal of this study was to determine whether variability in the 5-HTTLPR gene moderates the individual's biological response to stressful life events and influences development of the depressed condition. Genetic vulnerability to depression was operationalized by the degree to which the serotonin transporter gene (5-HTTLPR) was stimulated. A high level of serotonin is negatively correlated with the depressed condition. Data for this prospective-longitudinal study was gathered from a birth-cohort of 1,037 tracked from

age three to twenty-six. 17% of participants were homozygous for the short ("s") 5-HTTLRP allele (genotype s/s), 51% were heterozygous for the allele (geneotype s/l) and 31% were homozygous for the long ("l") 5-HTTLRP allele (geneotype l/l). Participants were asked to recall the number of stressful life events they experienced between the ages of twenty-one and twenty-six. Stressful experiences were considered across many facets of life, including employment, financial standing, health and interpersonal relationships.

Results of the study showed that, after experiencing four or more stressful life events, individuals homozygous for the "s" allele ("s/s") ranked significantly higher than individuals both with the "s/l" heterozygous genotype and the "l/l" homozygous genotype across all stress-related evaluations: self-reported depression symptoms, probability of major depression episodes, probability of suicide ideation/attempt and informant reports of depression. Individuals possessing the "s" allele, whether homozygous or heterozygous, demonstrated elevated amygdala activity in response to fear-inducing stimuli. The amygdala is a region of the brain responsible for memory formation and emotional response to internal and external stimuli. Considering both of these findings, it seems that the short ("s") allele of the 5-HTTLRP gene is correlated with an increased incidence of depression and heightened emotional reaction in response to fearful or stress-inducing events.

In addition to causing a variety of diseases, stress also plays role in reducing longevity by accelerating the cell "aging" process. Psychological stress is positively correlated with high levels of oxidative stress, low telomerase activity and shortened telomere length, all of which are factors known to increase morbidity. Telomerase is an enzyme that tacks the repeating nucleotide sequence "TTAGGG" onto the 3' end of chromosomes. This chromosome cap is a protective mechanism to preserve and stabilize the protein-encoding DNA of chromosomes during cell division. During mitosis, DNA polymerases are unable to fully replicate the telomeres, thus, the "caps" shorten with each successive cell division. Telomere length is indicative of an individual's biological "age," which is influenced both by chronological age (calendar years) and high levels of chronic stress. Oxidative stress, characterized by the body's inability to detoxify free-radicals and repair the associated damage, can also play a role in telomere shortening. Oxidative stress may contribute to the development of cancer, heart failure, heart attacks, Parkinson's diseases, and a variety of other serious illnesses. Antioxidants, by contrast, can slow the process of telomere shortening.

In a study published by the Proceedings of the National Academy of Sciences, 58 premenopausal mothers were examined on the basis on their environmental exposure to stress and their subsequent telomere length. The experimental group of participants included mothers of chronically ill children that required intense, constant care, while members of the control group cared for healthy children. The study controlled for the chronological age of participants.

As predicted, mothers in the experimental group reported significantly shorter telomeres, as a result of their high-stress environment. When controlled for chronological age and body mass index scores, the high-stress group was found to have much shorter telomeres (3,110 base pairs) when compared to the control group (3,660 base pairs). It is estimated that the telomere of an average, healthy adult decreases in length by 31 to 63 base pairs each year. A chronically stressed adult, in contrast, experiences a decrease in length of about 550 base pairs per year. This accelerated loss in telomere length corresponds to an additional biological "aging" of 9-17 years, when compared to non-stressed counterparts. There was also positive correlation observed between years spent in the role of caregiver and reduced telomere length, suggesting that the duration an individual is subjected to high levels of stress also contributes to accelerated cellular "aging." This study provides evidence for a causal relationship between prolonged environmental stress and decreased telomere length. Telomere shortening is correlated with expedited biological aging, both at the cellular and organismal level, and increased morbidity.

Understanding how the human body responds to stress at the biological and genetic level is essential, as nearly all people encounter stressors in their daily lives. As demonstrated through many clinical trials, long-term exposure to stressful environments can be detrimental to the individual's health, and serves as a risk factor for a variety of medical complications, such as cardiovascular disorders and depression. By studying individuals who suffer from stress-induced disorders, we can learn more about the role genetics plays in mediating the stress response, which will provide insight into how to treat such illnesses more effectively. Researchers have already identified several genes whose mutations are thought to be responsible for abnormal regulation of the stress response. Studies also show that stress can induce premature cellular aging, and accelerate conditions of morbidity. Genetic research gives us the tools necessary to promote health in populations who exhibit inappropriate responses to stress. Such research may have very positive effects on improving the quality of life and increasing longevity in all people.

Resources Consulted

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3181835/
http://stke.sciencemag.org/cgi/reprint/sci;301/5631/386.pdf
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