Alcohol Alert

National Institute on Alcohol Abuse and Alcoholism

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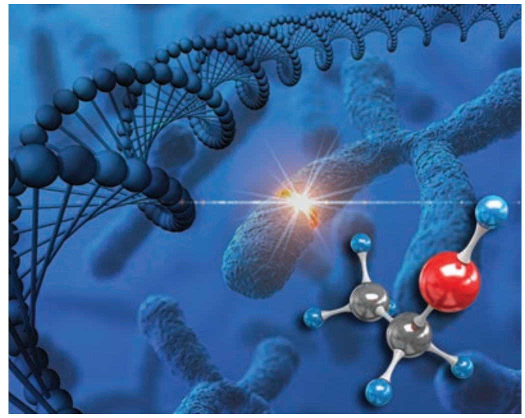
Epigenetics—A New Frontier for Alcohol Research



In today's modern age, with the entire human genetic makeup (the genome) decoded and genetic testing more and more common, most people understand that changes, or mutations, in genes can lead to death, disease, and disorder. But there is another way our genes play a role in physical dysfunction, and that occurs without direct alterations to DNA, the substance that makes up genes. Increasingly, researchers and physicians are exploring the concept of "epigenetics"— those changes that influence how genes are expressed but which do not involve physical changes in the DNA itself.

Epigenetics determines which genes the different cells in the body express at any given time, both during the development of an organism and during everyday functioning. During fetal development, for example, epigenetics ensures that the correct genes turn on at the right time in the right cells to allow tissues to develop properly. The process then continues to determine which genes turn on in which cells throughout life.

This rapidly emerging area of research suggests that age, environment, and exposure to drugs and other chemicals, including alcohol, directly affect epigenetics, altering normal epigenetic patterns and leading to abnormal expression or silencing of essential genes. The research also suggests that these epigenetic changes can, in some instances, be passed from one generation to the next. This *Alert* describes how alcohol influences epigenetics and how those influences may be associated with illness and disorders, including fetal alcohol spectrum disorders (FASD), cancer, liver disease and other gastrointestinal disorders, brain development, the body's internal clock, and immune function. It also explores therapies that might be developed to target the changes occurring through epigenetics.

Epigenetics Primer

Generally speaking, epigenetics provides each cell with its "identity." Every organism starts as a single cell. That cell divides, creating a mass of stem cells that, through a series of carefully orchestrated changes in gene expression, differentiate into the many types of cells needed to form the tissues and organs of the fetus. Epigenetics determines which genes are turned on in each type of cell, and maintains that pattern of gene expression—the cells' molecular identity—through unique alterations in how the DNA encoding each gene is packaged within the cell's nucleus. Genes in stretches of DNA that are tightly wound cannot be accessed by transcription factors and therefore are silent. Genes in stretches of DNA that are more relaxed and open are accessible and therefore active. The DNA of each cell type is packaged in a unique way that allows it to express the specific set of genes it needs to perform its physiological function.

To understand epigenetics, it's important to understand a bit about how DNA is packaged into the cell's nucleus. To fit, it must be supercondensed into chromosomes (Figure 1). This condensed DNA is called chromatin, which is made up of building blocks called nucleosomes, each consisting of a stretch of DNA wrapped around eight proteins called histones.

Anything that causes modifications to this chromatin structure can affect the first step of gene expression, which is called transcription and is the process that translates DNA into RNA and, in turn, RNA into proteins. Probably the best understood epigenetic mechanism that modifies chromatin is called methylation. During methylation, a quartet of atoms called a methyl group attaches to a gene at a specific point. There, it remodels the chromatin and affects how the gene is expressed. Methylation usually results in silencing of the gene. On the other hand, acetylation of chromatin (in which groups of molecules called acetyl are added) might help to relax tightly coiled chromatin, which can turn genes on. Many genes that are critical to prenatal and postnatal development will not function without methylation and are therefore vulnerable to diet and other environmental factors that affect methylation. Along with acting directly on genes, methylation, acetylation, and several other chemical reactions can also influence the chromatin structure by modifying histones.

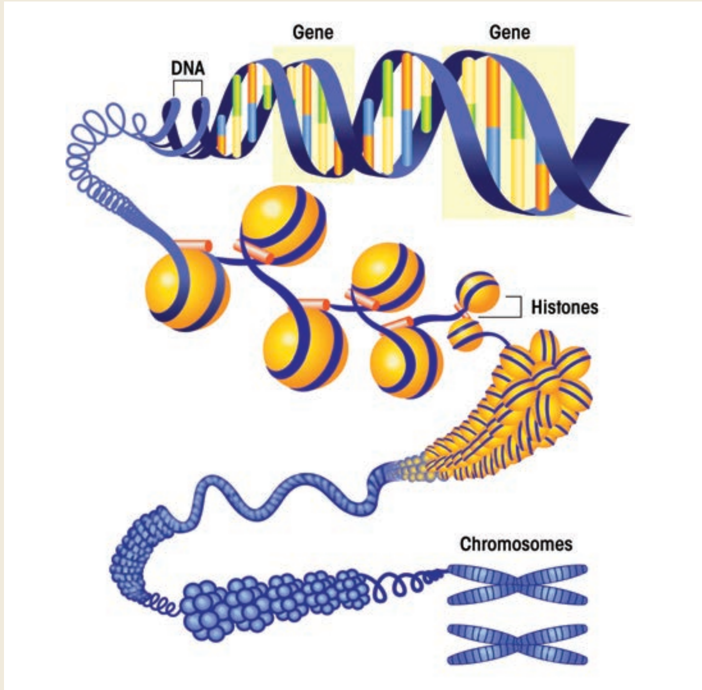


Figure 1 Chromosome structure. DNA is tightly wound around proteins called histones and packaged into cells' nuclei in the form of chromosomes. Genes are sections of DNA that, under the right circumstances, can be transcribed into proteins. Epigenetics determines which genes each cell transcribes at any given moment.

Another epigenetic mechanism that can affect which genes become active in cells works through a type of RNA called "non-coding RNA" (ncRNA). RNA is best known for its job as a middleman between DNA and proteins, acting as a template from which proteins are created. ncRNA does not code for proteins. Instead it acts as a switch, helping out during many cellular processes. Some small ncRNAs, called microRNAs (miRNAs), silence the expression of genes, either after they have been transcribed or during the process of transcription. Research shows that dysregulation of ncRNAs contributes to various diseases, including cancer and heart disease.[1]

How Alcohol Affects Epigenetics

Alcohol consumption leads to chemical changes within the body that can affect all of these epigenetic mechanisms.[1] For one, excessive alcohol consumption interferes with the body's ability to process and access a chemical called folate.[2] Folate is critical for methylation, which, as described above, plays a crucial role in epigenetics. So, in effect, alcohol consumption can lead to lower-than-normal methylation throughout the body, something called hypomethylation.

Research also finds that alcohol metabolism leads to histone modifications through a variety of pathways, including an increase in a substance called NADH, which is a byproduct of alcohol metabolism, and through production of reactive oxygen species (ROS), which are chemically reactive molecules that at high levels can damage cells.[1] Specifically, alcohol metabolism influences normal histone acetylation and methylation patterns, both of which can turn genes and proteins on and off, depending on the situation.[1]

The Consequences of Alcohol-Related Epigenetic Changes

Fetal Alcohol Spectrum Disorders

Women who drink during pregnancy put their developing fetuses at serious risk for a range of conditions collectively known as FASD.[3] Among other things, alcohol affects brain development in the fetus during critical periods of growth, leading to learning and behavioral problems. The exact effects of alcohol exposure during fetal development depend on the timing, amount, and duration of exposure as well as genetic susceptibility. Growing evidence from studies in rodents suggests that changes in chromatin structure caused by DNA methylation and histone modification contribute to FASD.[4] In addition, a small but rapidly expanding body of studies using animal models and cell cultures shows that alcohol influences miRNAs during fetal development and that this interaction likely plays a significant role in the development of FASD.[4]

In exploring how epigenetics contributes to FASD, researchers have also begun to investigate two complex enzymes that play a crucial role in cell differentiation during fetal development.[5] One, called polycomb protein, remodels chromatin to turn genes off; the other, called trithorax protein, remodels chromatin to turn genes on. Research suggests that exposure to alcohol may disrupt these two enzyme complexes, altering how cells differentiate during fetal development.[5] Indeed, alcohol's effect on these enzyme complexes might partially explain why alcohol consumption can have a dramatically different effect depending on the timing, amount, and duration of exposure. This is because the enzymes are more or less active at different times and in different tissues throughout development.

That said, alcohol's influence on prenatal epigenetics is difficult to study. These effects not only can vary depending on the type of cell being examined, but they also can be transient. Researchers are only beginning to explore the intertwined roles of different epigenetic mechanisms in brain development and how this process is affected by exposure to alcohol, resulting in FASD. The hope is that a better understanding of how alcohol influences epigenetics during fetal development will yield important clues for managing this wide spectrum of disorders.

Liver Disease and the Gastrointestinal Tract

Alcohol affects epigenetics on many levels within the gastrointestinal (GI) tract and liver, where the majority of consumed alcohol is broken down (i.e., metabolized) and cleared from the body.[6] As alcohol enters the liver, it sets off what could be described as a cascade of epigenetic changes that increase the risk of liver disease, liver cancer, and immunological problems. The changes include all of the mechanisms described above, including DNA methylation, histone modifications, and changes in the activity of various miRNA. The altered epigenetics caused by alcohol may play a role in alcohol-induced fatty liver and the progression of liver cancer, and may contribute to widespread changes in other organs.

In addition, alcohol-associated epigenetic changes may play a role in what researchers call organ "cross-talk" between the GI tract, the liver, and other organs. For one, epigenetic changes to genes involved in joining the cells lining the intestines[7] may be partially responsible for "leaky gut," which allows endotoxins to enter circulation and initiate liver damage. The response of the liver to alcohol endotoxins is complex and involves many types of cells. Alcohol causes epigenetic alterations in these cells that eventually lead to cross-talk among the organs of the GI tract.

Cancer

As suggested above, alcohol-related changes involved in epigenetics can be linked to the development of liver cancer. In particular, research suggests that some epigenetic changes can transform normal liver cells back into stem cells, which then can develop into liver cancer.[8] In addition, alcohol acts indirectly on a molecule called toll-like receptor [4] that, when disrupted, is involved in the development of liver cancer.[8] Alcohol consumption also influences miRNAs that control the expression of several genes associated with alcoholic liver disease.[8]

Alcohol's role in changing DNA methylation patterns, leading to hypomethylation, may be one of the main routes between alcohol consumption and liver cancer as well as other types of alcohol-associated cancers, including breast cancer and cancers of the upper respiratory and digestive tract.[9] Many studies show that DNA methylation patterns contribute to both tumor initiation and progression. In one study of 609 patients, those who drank excessively were at increased risk of colon cancer, which in turn was associated with decreased DNA methylation.[10] Studies show a similar pattern for breast cancer[11] and cancers of the upper respiratory and digestive tract,[12] including cancers of the mouth, throat, and voice box (larynx).

Brain

Alcohol's epigenetic effects within the brain are complex and intertwined. But increasing evidence suggests that they result in adaptations within the brain that ultimately influence addictive behaviors, including tolerance and alcohol dependence.[13]

As seen in other disorders, changes in DNA methylation are one of the epigenetic changes in the brain caused by chronic alcohol consumption.[14,15] Interestingly, researchers see an overall decrease in DNA methylation within the brain, but also increased methylation at the promoter site of several specific genes. This multifaceted methylation pattern is similar to one seen in studies of liver cancer.

Other studies find evidence that alcohol consumption affects histone acetylation in the brain and that this is associated with alcohol-related behaviors, such as withdrawal-related anxiety,[16] alcohol consumption,[17] rapid tolerance,[18] and conditioned place aversion (a laboratory method for assessing how motivated an animal is to obtain alcohol).[19]

Although researchers still are piecing together the details, findings to date suggest that epigenetic changes in gene expression induced by alcohol consumption may underlie the brain pathology and adaptations in brain functioning associated with alcohol abuse and alcohol dependence and may contribute to alcohol relapse and craving.[13]

Circadian Disruption

Circadian rhythms are not just a feature of biology that affects sleep cycles. Individual cells, tissues, organs, and whole organisms have internal clocks that allow them to synchronize their functions to a 24-hour day.[20] These circadian rhythms help organisms function most efficiently by controlling the sleep/wake cycle, body temperature, hormone secretion, intestinal function, glucose metabolism, and immune function, and allow them to anticipate things such as food availability. Alcohol consumption disrupts circadian rhythms,[20] which, in turn, promotes the development and/or progression of a wide variety of diseases, including inflammatory, metabolic, and alcohol-associated disorders.

Epigenetic Mechanisms

- **DNA Methylation:** A biochemical process that attaches a methyl group to a specific spot on DNA. DNA methylation acts to lock genes in the "off" position. Chronic alcohol consumption leads to lower-than-normal methylation, or "hypomethylation."
- **Histone Modification:** To condense itself enough to fit inside the nucleus of cells, DNA wraps around proteins called histones (Figure 1). Histones can be modified in many ways, and these modifications can turn genes both on and off, depending on the situation. Alcohol metabolism leads to histone modifications through a variety of pathways.
- **microRNA Alterations:** Some small non-coding RNAs, called microRNAs (miRNAs), silence the expression of genes, either after they have been transcribed or during the process of transcription. Alcohol changes the activity of various miRNA during fetal development and in adult tissues.

Both circadian disruption—for example, long-term shift work—and alcohol consumption affect epigenetic mechanisms and are associated with a wide variety of health outcomes, including cancer and immune system dysfunction.[20] Alcohol sets in motion a cycle of epigenetic changes. It influences the circadian rhythm by changing how circadian-clock genes get expressed. Then, disrupted circadian rhythms negatively influence immune function and further promote alcohol consumption, which leads to further circadian-rhythm disruption. A better understanding of how circadian rhythms influence bodily functions and how environmental factors such as alcohol use influence these processes is vital.[20]

Immune System

Alcohol enhances the risk for developing several serious medical conditions related to immune system dysfunction, including acute respiratory distress syndrome (ARDS), liver cancer, and alcoholic liver disease (ALD).[21] In addition, binge and chronic drinking put people at higher risk for many infections and can advance the progression of HIV infection by weakening the body's ability to fight disease.

Epigenetic mechanisms can be traced to many of these processes. For example, alcohol-induced epigenetic variations alter the developmental pathways of several types of immune cells, including granulocytes, macrophages, and T-lymphocytes.[21] Through these and other mechanisms, the epigenetic changes promote exaggerated inflammatory responses. Epigenetic mechanisms may also underlie alcohol's ability to interfere with the barrier functions of the gut and respiratory systems, which also contribute to the heightened risk of infections.

Targeting Epigenetics for Therapy

Better understanding of the link between alcohol consumption, epigenetic changes, and illness and disease could lead researchers to develop medications or therapies for prevention or treatment. For example, a deeper understanding of the molecular mechanisms that lead to altered DNA methylation patterns caused by alcohol consumption may allow researchers to devise effective therapies to combat them.[9] In addition, researchers can now analyze DNA methylation patterns for the entire human genome. This work could yield comprehensive maps of DNA methylation changes in alcohol-associated cancers. Those maps then could potentially be used to develop pharmacological treatments that target epigenetic markers as well as to develop new markers for cancer detection and prognosis.[9]

As scientists learn more about alcohol's detrimental effects on the epigenetic processes involved in regulating the immune system, this research may lead to new medications for preventing or treating infections and other problems.[21] Already, epigenetic research has been used to develop drugs that target epigenetic "master regulators," which can affect the expression of multiple genes by influencing chromatin.[13] These drugs are emerging as potential therapeutics for neurodegenerative disorders and drug addiction.

Conclusion

Alcohol clearly influences epigenetic changes, both transiently and permanently, and those changes influence a variety of cells and organ systems throughout the body. As researchers begin to untangle the exact nature of alcohol's interactions with epigenetics, they will be able to design better medications to treat or alleviate a wide range of alcohol-related disorders, including FASD, alcohol addiction, cancer, and organ damage.

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