Current Addiction Reports (2020) 7:446–452
https://doi.org/10.1007/s40429-020-00331-x

ALCOHOL (A HEINZ & N ROMANCZUK-SEIFERTH, SECTION EDITORS)

Prospects of Genetics and Epigenetics of Alcohol Use Disorder

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Published online: 15 September 2020

Abstract

Purpose of Review In this study, we illustrate recent findings regarding the genetics and epigenetics of alcohol use disorder (AUD). We further outline the future direction of genetic and epigenetic research in AUD.

Recent Findings Recent genome- and epigenome-wide studies allow new insight into genetic and epigenetic variation associated with AUD. The largest EWAS of AUD so far/to date found evidence for altered glucocorticoid receptor regulation. Longitudinal studies provide insight into the dynamics of the disease. Analyses of postmortem brain tissue reveal the impact of chronic alcohol consumption on DNA methylation in the brain.

Summary Genetic and environmental factors—mediated via epigenetic mechanisms—play an important role in AUD. Although knowledge of the biological underpinnings of AUD is still limited, ongoing research will ultimately lead to the development of biomarkers for disease classification, course of disease, and treatment response to support personalized medicine in the future.

Keywords Alcohol use disorder · Addiction · Genetics · Epigenetics · Neurobiology · Personalized medicine

Introduction

The recent WHO global status report on alcohol and health estimates that alcohol is consumed by more than half of the population in both the USA and Europe. Harmful use of alcohol caused about 3 million deaths worldwide in 2016, which are 5.3% of all deaths [1]. In 2010, the costs of excessive drinking in the USA amounted to 249 billion US dollars, which is 46 billion dollars more than in 2006 [2]. Epidemiologic data on the DSM-5 classification of alcohol use disorder (AUD) in the years 2012 and 2013 in the USA showed that the 12-month and lifetime prevalences of AUD were 13.9% and 29.1%, respectively [3]. Compared with similar data from 2002, alcohol consumption among US adults has increased over the course of a decade [4]. Prevalence of AUD in countries of the European Union, Iceland, Norway, and Switzerland was estimated at 23 million affected people in 2010, equivalent to 3.4% among people 18–64 years of age [5]. A meta-analysis showed that mortality is significantly higher in persons with alcohol dependence, but a reduction in alcohol intake can decrease this risk [6]. The increased risk of mortality associated with AUD might be due to the genetic predisposition of individuals who develop AUD and a direct result of having AUD and/or increased alcohol intake associated with AUD. While genetic predisposition has a greater effect on mortality early in life, the direct results of having AUD become more important later in life and over the course of the disease [7].

AUD can be characterized by several criteria such as intake in spite of harmful consequences, loss of control over alcohol consumption, drug craving, tolerance development, and the manifestation of withdrawal symptoms [8]. On a neurobiological level, the process of developing AUD includes sensitization of dopamine release within the reward system, which has been shown in several animal models [9, 10], (for the sensitization concept [11]), the reorganization of striatal loops, which is supposedly associated with the habituation of drug intake [12, 13], and neuroadaptation within the limbic and

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This article is part of the Topical Collection on *Alcohol*

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stress systems [14, 15]. Studies have found that genetic variants associated with alcohol dependence are involved in alcohol metabolism as well as in dopaminergic and serotonergic transmission [16•]. While past research has mainly focused on the biological changes involved in the development of AUD, the complex role of environmental factors such as psychosocial stress is subject of more recent studies: In an adoption study, the authors found that AUD in offspring was associated with AUD and other diseases in not-lived-with biological parents as well as in stepparents, underlining that genetic and environmental risk factors act additively on the risk of developing AUD [17]. Effects of environmental factors may be mediated by epigenetic mechanisms [16•]. In the sections below, we present and discuss recent advances in research on the genetics and epigenetics of AUD. We will first give a short introduction to DNA methylation, the most extensively studied epigenetic mechanism. This introduction will be followed by an outline of epigenetic mechanisms investigated in association with alcohol use. We will then present recent findings of genetic studies in the field. Finally, we will discuss the prospects of research on genetic and epigenetic factors in AUD disease etiology.

Epigenetic Mechanisms

Environmental factors may have an effect on the development, course, and treatment of diseases via epigenetic mechanisms (e.g., [18, 19]). Epigenetic mechanisms describe a series of biochemical processes that can alter the phenotype of an individual without altering the DNA sequence. One of the best-studied epigenetic mechanisms is DNA methylation, which can alter gene function [20]. Methylation refers to the addition of a methyl group to the nucleobase cytosine at the 5′ position of the cytosine pyrimidine ring in cytosine-guanine (CG) dinucleotide sites. Genomic regions where CG dinucleotides occur at a high frequency of more than 200 base pairs in a row are referred to as CpG islands. As CpG islands usually occur near the promoter region or at the start site of genes, their methylation plays an important role in gene regulation.

Epigenetic modulation of gene transcription and consecutive long-term changes in gene function have been suggested to account for a large part of phenotypic variation. Although it was initially assumed that epigenetic modifications are "fixed" very early in development, evidence now suggests that this is not strictly the case. For example, in animals, individual responses to stress in adulthood can be altered by adversity in early life [21]. Maternal rats' caring behavior in the first week after birth has been shown to alter the offspring's DNA methylation at the glucocorticoid reporter gene in the hippocampus, a brain region mainly involved in learning and habituation processes. Animals with caring mothers showed higher glucocorticoid receptor levels, had reduced corticosterone, and were less anxious compared with the offspring of less-caring mothers [22]. In a translation from previous findings in animal studies [23, 24], it has been shown in humans that childhood abuse [25, 26] and parental separation [27] have an effect on the epigenetic regulation of genes involved in stress-regulation [25, 26], immune response, and cellular signaling systems relevant for neural communication and brain development and functioning [27]. In another animal study, rats exposed to alcohol during fetal development ($n = 6$) showed altered methylation levels of proopiomelanocortin gene (*POMC*) in the arcuate nucleus, which is relevant for both stress and fear management [28].

Methylation levels can also be altered by interventions such as alcohol withdrawal [29] and psychotherapy for stress prevention [19], thus giving insight into the molecular epigenetic mechanisms underlying therapy response.

In summary, epigenetic modifications such as DNA methylation regulate gene expression, ultimately leading to alterations in phenotype. Epigenetic mechanisms can explain some aspects of the role of environmental factors in disease etiology and shed light on the underlying mechanisms of therapy response.

Epigenetic Factors

In the following paragraphs, we will outline recent advances in epigenetic studies for different aspects of AUD. The findings are presented in order of the type of study: peripheral blood sampling, longitudinal sampling, and finally brain tissue sampling. The advantages and limitations of each method for studying epigenetic markers are discussed.

The most common approach to identify the epigenetic mechanisms involved in AUD is to compare methylation profiles of cases to those of healthy controls in peripheral blood samples. To date, the largest epigenome-wide association study (EWAS) of alcohol consumption was conducted in over 13,000 subjects from different cohorts and found 328 differentially methylated CpG sites in European- and 165 in African-ancestry samples. The methylation levels of 144 differentially methylated CpG sites provided substantial discrimination between current heavy drinkers and non-drinkers of European ancestry [30]. Further, in a subset of the European ancestry sample, the amount of alcohol exposure was associated with methylation patterns in the γ-aminobutyric acid-A receptor (*GABA*) A and B genes that are related to the development of addiction [30, 31]. Differentially methylated CpG sites were also identified in promoter regions of genes relevant to immune functioning [30]. An EWAS in 1,135 subjects analyzing the association between methylation levels and the amount of alcohol consumed found 64 CpG sites associated with alcohol intake [32]. Six of the 64 CpG sites that have been previously reported to be associated with AUD [30],

liver function [33], body mass index, and lipid metabolism [34] could be replicated [32].

An EWAS of 18 twins discordant for AUD showed an association between AUD and methylation levels within the promoter region of 3′-protein-phosphatase-1G gene (*PPM1G*), a gene related to cell stress response [35]. This finding was validated in epigenome-wide data on binge drinking in a cohort of 499 adolescents [35]. Additionally, in this cohort, methylation levels were associated with the intensity of a reward signal from a functional magnetic resonance imaging (fMRI) scan, demonstrating the relevance of epigenetic factors for neurobiological function [35]. In another fMRI study, the authors tested 383 heavy drinkers with an fMRI alcohol reward paradigm and found an association of dopamine D2 receptor gene (*DRD2*) promoter methylation level with signal change in the striatum during the presentation of alcohol cues as well as the severity of AUD [36]. These findings suggest an effect of methylation patterns associated with addiction-relevant behavior on brain areas functionally relevant for the development and maintenance of addiction-relevant behavior.

Longitudinal studies can shed further light on the biological mechanisms underlying the course of illness [for AUD] and the effectiveness of interventions. In a longitudinal study of 165 female participants, different degrees of alcohol consumption over the past 6 months were found to be associated with two loci reaching epigenome-wide significance [37]. Another EWAS compared methylation patterns in 24 patients before and after a 3-week detoxification program and 23 controls [38]. Here, 59 CpG sites were differentially methylated between patients and controls before entering the treatment program and 48 CpG sites were differentially methylated comparing patients before and after treatment [38].

Massive alterations of methylation patterns were found between 99 male AUD patients and 95 controls at the time of acute withdrawal as well as after 14 days of withdrawal [39]. Especially pathways related to immune function were implicated, which is in line with previous studies linking immune function to alcohol consumption and withdrawal (e.g., [40]). Interestingly, in this study, differences between patients and controls were less pronounced after withdrawal, suggesting that methylation levels may have reverted back to normal during treatment. Those differences that remained between patients and controls after withdrawal may indicate genes and pathways implicated in alcohol addiction itself or the vulnerability to become addicted. In a sample of 69 detoxified patients followed up for 12 months, no difference in methylation levels between patients who relapsed and those who abstained was found [41]; moreover, no change over time was found.

In human studies, DNA methylation changes in peripheral blood are most commonly investigated. Samples from other tissues, which are likely to be altered by long-lasting alcohol consumption (e.g., brain, liver) are rare and difficult to obtain. There is evidence for some concordance between peripheral blood and brain tissue concerning their DNA methylation patterns, with correlation coefficients estimated to range between 0.33 and 0.40 [42•]. Still, the analysis of DNA methylation patterns in human brain tissue is of particular interest, as the brain is likely involved in the development and maintenance of AUD.

One of the first EWAS in human postmortem brain tissue investigated DNA methylation differences in the prefrontal cortex (PFC; Brodmann Area 9) of 23 subjects with AUD (7 female) and 23 age- and sex-matched controls [43]. Analyses were conducted separately for male and female subjects. A total of 1,812 differentially methylated CpG sites were found to be associated with AUD case/control status in males. No observed differences in methylation between cases and controls were present in female subjects, indicating a possible effect of sex. Furthermore, 22 co-methylation modules (i.e., groups of co-methylated CpGs, clustered by pairwise correlations) were associated with AUD in males. The largest module was enriched for the biological processes: cell projection organization, cell projection morphogenesis, and neuron development. Of note, genes identified to be associated with substance use phenotypes in genome-wide association studies (GWAS) were overrepresented in the two largest modules. A more recent investigation of DNA methylation levels in tissue from Brodmann Area 9 found two differentially methylated regions, one annotated to the gene that codes for the discs large-associated protein 2 (*DLGAP2*) [44]. *DLGAP2* encodes membrane-associated guanylate kinases relevant for the organization of synapses involved in neuronal cell signaling. In another study, over 400 differentially methylated CpG sites were identified in precuneus brain samples of 49 subjects (11 female) and 47 controls (12 female) [45].

In a recent EWAS, DNA methylation was investigated in the PFC (Brodmann Area 10) of 25 pairs of AUD cases and controls [46]. While the authors identified a large number of nominally significant differentially methylated sites, they focused on genes related to stress adaptation, including the nuclear receptor subfamily 3 group C member 1 gene (*NR3C1*). They found chronic alcohol consumption to be associated with increased methylation levels of *NR3C1* and decreased expression of the related protein as well as alterations in the expression of several other stress-responsive genes in the PFC of AUD cases. These findings underline the notion that environmental factors including stress exposure are one of the key factors explaining the development and continuation of behavior related to alcohol use disorders [35, 46, 47].

Moreover, in a cross-tissue and cross-phenotype study, the authors demonstrated an association between DNA methylation levels in the promoter region of the proprotein convertase subtilisin/kexin 9 gene (*PCSK9*), a gene involved in cholesterol metabolism, and AUD [48]. Findings from the three discovery set analyses in human brain tissue were replicated in blood and liver tissue as well as in animal models [48]. In the largest

EWAS of AUD so far, evidence for an altered glucocorticoid regulation in AUD emerged [49••]. The EWAS was conducted in six independent cohorts on subjects with AUD, and results were replicated across multiple tissues. AUD was associated with DNA methylation in genes associated with immune response and glucocorticoid signaling. A marker that has been consistently associated was the growth arrest–specific five gene (*GAS5*), which has been implicated in the regulation of glucocorticoid receptors [49••].

However, there are several challenges concerning postmortem studies of brain tissue: due to cell type heterogeneity, comparability of data retrieved from a given tissue might not be given. Also, in the context of AUD, there are far more male postmortem brain samples available (e.g., New South Wales Tissue Resource Centre (NSW TRC)). Therefore, it is difficult to identify underlying biological mechanisms that differ considerably between males and females. Future studies on the single-cell level will help to analyze methylation patterns in more detail. Because of the rarity of this type of tissue and the high number of samples needed to reach adequate statistical power, we conclude that available datasets need to be combined with in silico brain banks and meta-analytical approaches.

In summary, there is accumulating evidence for the association of epigenetic markers with AUD. However, a causal relationship has yet to be established.

Genetic Factors

Apart from epigenetic factors, genetic factors contribute to AUD. The heritability of AUD is typically estimated to be around 40% [50]. Genetic factors also play a role in the course and severity of addiction [51]. Large GWAS and meta-analyses have identified genes and pathways implicated in AUD [52, 53, 54•, 55]. The most recent GWAS identified 29 risk variants associated with problematic alcohol use [56••]. These studies could also corroborate known candidate genes, e.g., in neurotransmitter systems such as the dopaminergic, cholinergic, serotonergic, noradrenergic, glutamatergic, and GABAergic systems. These insights facilitate a better understanding of the molecular mechanisms underlying AUD.

Polygenic methods can be used to analyze the aggregated genetic effects of all markers associated with an outcome. A polygenic risk score (PRS) is the weighted sum of all known risk alleles for a specific outcome calculated using the information of large GWAS [57]. It is a single value that reflects an individual's overall genetic risk for, or inherited susceptibility to, a disease. Because polygenic risk scores are calculated based on the summary statistics of GWAS, the features of the GWAS (e.g., sample size, ancestry, explained SNP-heritability) also influence the PRS. PRS are associated with disease severity and the course of illness for a variety of psychiatric disorders (e.g., [58]). With respect to AUD, past studies have shown that aggregated genetic risk measured by PRS is associated with alcohol consumption as well as alcohol dependence [59, 60]. Using polygenic methods to analyze genetic correlations, relations were detected between problematic drinking and other substance use and psychiatric traits, as well as between alcohol-related traits measured longitudinally by using Alcohol Use Disorder Identification Test-Consumption (AUDIT-C) scores [61] and AUD.

Furthermore, there are efforts to investigate these associations in a more refined way by examining the influence of a specific genetic predisposition, e.g., scores only including genes from dopaminergic, serotonergic, and glutamatergic pathways and/or PRS for related domains such as cognition, stress reactivity, and sensation seeking on disease mechanisms involved in substance use and addiction [62]. An area of particular interest that has not yet been fully exploited is the association of these PRS with dimensions of observable behavior and neurobiological measures as measured with research domain criteria (RDoCs, e.g., impulsivity, negative affect, cognitive control) [63].

In other areas of medicine such as cardiology, PRS have been used to successfully identify high-risk subgroups of patients who benefit more from specific therapies [64]. The reliable use of PRS requires sufficient GWAS summary statistics, and larger sample sizes are necessary. It is common that large GWAS only include European samples [56••]. While there have been substantial efforts to perform cross-ancestral GWAS [52, 54•, 61], especially in the field of substance use disorders, prediction of the genetic risk for AUD remains less accurate for non-European populations.

Prospects of Genetic and Epigenetic Factors in AUD

Recent advances in genetic and epigenetic research of AUD provide insight into possible mechanistic underpinnings and potential biomarkers for disease classification, course of disease, and treatment response. To date, however, knowledge of the molecular basis, i.e., the genomic and epigenomic processes underlying addiction, remains fragmentary. Large-scale and/or longitudinal studies with an intensive characterization of participants from multiple populations are necessary to further identify the underlying biological processes that contribute to the risk of alcohol addiction and recovery. Previous efforts to investigate the genetic markers of AUD for clinical use have been largely limited to studies of single markers in pharmacological applications. So far, there is a lack of evidence regarding genetic predictors of medication efficacy (e.g., [65]). Also, the prediction accuracy of aggregated genetic markers, such as PRS, is still limited. Larger GWAS are necessary to enhance the power of the discovery sets.

In general, developing and establishing robust biomarkers and algorithms to predict diagnoses and treatment response necessitates the comprehensive integration of intensive genetic, epigenetic, transcriptomic, metabolomic, microbiomic, and phenotypic longitudinal data [66]. As sample sizes increase, machine learning approaches can be used as a promising data-driven way to analyze large-scale multi-omics data. New developments such as massively parallelizing applied algorithms in combination with the development of more powerful computing hardware make it possible to process such data within a reasonable timeframe and will eventually lead to applicability for individualized interventions and prevention.

Moreover, clinicians and patients must be well informed about this extremely complex subject involving the importance of genetics and environmental factors. The participation of patient representatives, clinicians, geneticists, and bioinformaticians is essential in this process.

Funding Open Access funding provided by Projekt DEAL.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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