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Drinking beyond a lifetime: New and emerging insights into paternal alcohol exposure on subsequent generations

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ABSTRACT

Alcohol-use disorder (AUD) is prevalent and associated with substantial socioeconomic costs. While heritability estimates of AUD are ~50%, identifying specific gene variants associated with risk for AUD has proven challenging despite considerable investment. Emerging research into heritability of complex diseases has implicated transmission of epigenetic variants in the development of behavioral phenotypes, including drug preference and drug-induced behavior. Several recent rodent studies have specifically focused on paternal transmission of epigenetic variants, which is especially relevant because sires are not present for offspring rearing and changes to offspring phenotype are assumed to result from modifications to the sperm epigenome. While considerable interest in paternal transmission of epigenetic variants has emerged recently, paternal alcohol exposures have been studied for 30+ years with interesting behavioral and physiologic effects noted on offspring. However, only recently, with improvements in technology to identify epigenetic modifications in germ cells, has it been possible to identify mechanisms by which paternal ethanol exposure alters offspring behavior. This review presents an overview of epigenetic inheritance in the context of paternal ethanol exposure and suggests future studies to identify specific effects of paternal ethanol exposure on offspring behavior and response to ethanol.

Introduction

A large number of recent studies have challenged traditional views of Mendelian inheritance by showing that offspring phenotype can be modified by parental exposure to nutritional changes, stress, drugs of abuse, and other factors. These studies have implicated heritability of germ-line encoded epigenetic variants in mediating these effects. Converging evidence has also shown that ethanol is an epi-mutagen in several tissue types, including germ cells. Based on known heritability of alcohol-use disorder (AUD) and difficulty in identifying specific gene variants associated with AUD, studying heritability of ethanol-induced epigenetic modifications and their impact on ethanol-related behaviors in subsequent generations has the potential to advance our understanding of the etiology of AUD and to elucidate new biomarkers for AUD.

Heritability of alcohol-use disorder

The lifetime prevalence of AUD is 30% in the United States (Hasin, Stinson, Ogburn, & Grant, 2007), highlighting the likelihood of significant genetic and environmental differences among such a large cohort. This diversity is further complicated by the wide spectrum of alcohol (ethanol) consumption among humans, contributing to varied thresholds for tolerance and dependence among those who meet DSM-V criteria for AUD (American Psychiatric Association, 2013). Heterogeneity has made drug development for AUD challenging, since factors promoting pathological alcohol consumption are likely to be complex and varied between subjects. Despite this diversity, twin and adoption studies consistently find that AUD has a heritability of ~50% (Prescott & Kendler, 1999; Young-Wolff, Enoch, & Prescott, 2011; Ystrom, Reichborn-Kjennerud, Aggen, & Kendler, 2011), indicating transmission of risk alleles from parents to offspring independent of environment.

Based on the known heritability of AUD, studies have extended to heritable physiological markers that may account for increased risk for AUD, including level of response to ethanol and ethanol

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metabolism. An early meta-analysis of several studies found that sons of alcoholics had decreased subjective response to a low dose of ethanol (Pollock, 1992). Utilizing subjects from the Australian Alcohol Challenge Twin Study, a more recent study quantified heritability of subjective response to a low dose (0.75 g/kg) of ethanol as ~60% in both men and women (Heath et al., 1999). These studies indicate that decreased level of response to ethanol, which is associated with increased risk for AUD (Schuckit, 1994), may be a component of AUD heritability. Observer-measured studies of sensitivity to ethanol have focused on heritability of static ataxia (body sway) after ethanol consumption and have generally noted lower increases in body sway after ethanol in children of alcoholics (Lex, Lukas, Greenwald, & Mendelson, 1988; Schuckit, 1985). Physiological measures of response to ethanol also show evidence for heritability. One study noted that sons of alcoholics had lower plasma cortisol levels after receiving a 1.1 mL/kg dose of 95% ethanol (Schuckit, Gold, & Risch, 1987). Notably, several groups have studied the p300 event-related potential, an electroencephalographic finding associated with attending to a stimulus (Polich, 2012), in people with a family history of alcoholism. These studies have found decreased p300 amplitude in children of alcoholics and have inversely correlated p300 amplitude with future risk for AUD (Begleiter, Porjesz, Bihari, & Kissin, 1984; Costa et al., 2000; Hesselbrock, Begleiter, Porjesz, O'Connor, & Bauer, 2001).

While complex ethanol-related behaviors are difficult to associate with heritable genetic variants, changes in ethanol metabolism have been linked to mutations in single genes. In particular, heritable variants of alcohol (ADH) and acetaldehyde (ALDH) dehydrogenase enzymes are known to be modifiers of alcohol consumption and risk for AUD. Polymorphisms inactivating the ALDH2 gene have been found almost exclusively in Asian populations and are associated with decreased risk for developing AUD (Higuchi, Matsushita, Murayama, Takagi, & Hayashida, 1995; Li, Zhao, & Gelernter, 2012; Thomasson et al., 1991). ADH1 and ADH7 single-nucleotide polymorphisms (SNP) have been associated with alcohol metabolism and consumption in European and African populations (Bierut et al., 2012; Birley et al., 2008). ADH1 polymorphisms also modulate vulnerability to fetal alcohol syndrome disorders (FASD) in fetuses exposed to ethanol (Warren & Li, 2005).

Increasing evidence implicating heritability of ethanol response and metabolism in modifying risk for AUD has led to a broader search for genetic variants associated with AUD. These studies have utilized population-level genetics to measure this association in large numbers of subjects across millions of SNPs. However, while such efforts have identified several potential loci that modify AUD risk, they have also left many questions unanswered regarding how alcohol-related behaviors are inherited. In particular, one recent genome-wide association study (GWAS) did not identify any SNPs significantly associated with alcoholism risk and estimated that all of the SNPs studied accounted for only 0.1% of the genetic risk for developing alcoholism (Heath et al., 2011). Other groups have found SNPs significantly associated with AUD using GWAS but later failed to replicate their results (Bierut et al., 2010; Treutlein & Rietschel, 2011). More recent meta-analyses and expanded studies have identified novel SNPs significantly associated with AUD (Gelernter et al., 2013; Wang et al., 2013), though differences in the SNPs discovered between studies suggest they may not be meaningful across the entire population. "Missing heritability" is a recent concept that refers to the inability of GWAS to uncover risk alleles that explain a substantial portion of the heritability of complex diseases. While technical issues and the contribution of rare genetic variants may be masking these alleles (Manolio et al., 2009), it is also possible that heritable variants outside the DNA sequence, known as "epi-alleles", may be contributing to complex phenotypes such as those mediating susceptibility to AUD.

Epigenetic modifications

Epigenetics deals with a broad group of processes that drive stable states of gene expression without changing nucleotide sequence. These processes are mediated by a diverse set of epigenetic modifications of DNA, including histone modifications and the proteins that create them, covalent modifications to DNA, large and small non-coding RNAs, and proteins that interact with DNA and its epigenetic modifications (Watanabe et al., 2011). The complement of epigenetic modifications associated with a genome is termed the "epigenome". Epigenetic mechanisms are the primary drivers of transcriptional regulation, and allow a single genome to give rise to the hundreds of stable cell lineages within an organism; thus, the genome of an organism is associated with many epigenomes in distinct cell types, and these epigenomes control the expression of genes that determine the cell's phenotype. The environment can dramatically impact epigenetic modifications (Feil & Fraga, 2012), including those within the germ line. Since some epigenetic modifications are mitotically and meiotically heritable (Reik, 2007), germ-line encoded epigenetic modifications may function as "epi-alleles" if they alter development and/or phenotype in the next generation.

The basic unit of the eukaryotic epigenome is the nucleosome, which consists of ~147 base pairs of DNA wrapped around a core of 8 histone proteins. All histone proteins are rich in basic amino acids that carry a net positive charge, imparting a strong affinity for the negatively charged DNA phosphodiester backbone. The affinity between histones and DNA is critical for regulation of gene expression and is altered by covalent modifications to histone N-terminal tails (Smith & Shilatifard, 2010). For example, acetylation of lysine residues neutralizes the positive charge on lysine's ammonium group, reducing its affinity for DNA; weaker histone–DNA interactions increase accessibility of DNA to transcription factors, which recruit RNA polymerase to initiate transcription (Lee, Hayes, Pruss, & Wolffe, 1993). Histone modifications are catalyzed by a diverse group of histone-modifying enzymes and are rapidly reversible, so that they are a key mechanism of cellular adaptation to the environment (Smith & Shilatifard, 2010). However, their role in gene regulation is complex, as recent studies have identified over 100 post-translational modifications to histones and the function of most of these is unknown (Tan et al., 2011).

The primary covalent modification to DNA in vertebrates is the methylation of cytosine preceding guanine (the CpG dinucleotide). CpGs occur much less frequently throughout the genome than would be expected by chance, most likely due to deamination of methylcytosine to thymine (Schorderet & Gartler, 1992). However, near transcriptional start sites of most mammalian genes, the density of unmethylated CpG dinucleotides is increased at regions known as "CpG islands" (Bird, Taggart, Frommer, Miller, & Macleod, 1985; Gardiner-Garden & Frommer, 1987). CpG islands show tissue-specific patterns of methylation; only ~8% are hypermethylated in most cell types and methylation is associated with transcriptional silencing of associated genes (Illingworth et al., 2008). Importantly, DNA methylation at CpG islands may indicate a relatively stable mechanism of transcriptional repression. Several studies have now shown that nucleosome repression, through histone methylation and induction of the polycomb repressive complex, precedes DNA methylation and that DNA methylation may "lock" gene promoters into a repressive state (Gal-Yam et al., 2008; Jones, 2012; Kass, Landsberger, & Wolffe, 1997). However, though DNA methylation is a critical component of gene regulation and suppression of retroviral elements (Jones, 2012), mechanisms for induction of DNA methyltransferases (DNMT) and pathways for demethylation are still poorly understood (Kohli & Zhang, 2013).