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The complex interplay of genetics, epigenetics, and environment in the predisposition to alcohol dependence

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SUMMARY

Alcohol dependence is a major global problem, associated with lower quality of physical and mental health, higher mortality and an enormous familial and social cost. Prevention strategies and treatment of this condition are therefore crucial. Success of psychosocial programs and pharmacological treatments has been frequently reported, but a better understanding of the etiology of this chronic disease is needed. For this purpose, the identification of associated factors in different populations is of great significance.

It has been clearly demonstrated by twin and adoption studies and supported by animal models that both genetic and environmental components play a substancial role in alcohol dependence. Heritability estimates range from 40 to 60%, depending on the specific analyzed sample.

Several coexisting genetic variants in each affected individual, rather than a single gene transmitted in a Mendelian manner, may be the rule in alcohol dependence. Similarly, many environmental factors can increase susceptibility, and because of their diversity, they do not have to be the same in every affected person. Environmental contribution may be linked to epigenetics, which refers to chemical processes that can modify gene activity without changing the sequence of DNA. In humans, the most stable epigenetic process is the union of a methyl group (one carbon atom surrounded by three hydrogen atoms) to cytosines in DNA. Other epigenetic mechanisms are modifications to nuclear proteins called histones, which alter the way DNA is packed. Moreover, non-protein coding RNAs such as microRNAs have been associated with the development of alcohol dependence. MicroRNAs could work as epigenetic intermediaries that allow ethanol to affect complex and divergent developmental mechanisms, which is added to the effect of DNA methylation, histone acetylation, and other epigenetic modifications.

Most reasearch points to an association between alcohol dependence and genes related with alcohol metabolism, with neurotransmission of dopamine, GABA, serotonin, glutamate, endogenous opioids, and cannabinoids, signal transduction within the mesolimbic dopamine reward system, and stress response system, among others.

During pregnancy, there are several non-genetic factors that may have an important impact on vulnerability to alcohol dependence. Given that the Central Nervous System is developing throughout the entire pregnancy and that alcohol consumed by the mother can reach the fetus through the placental barrier, the brain of a baby is always vulnerable to harm caused by alcohol exposure. Children born to alcoholic mothers may inherit genetic susceptibility variants but at the same time they may be exposed to early effects of ethanol. Heavy alcohol exposure during pregnancy has been associated with mental retardation, epilepsy, attention deficit/ hyperactivity disorder, learning disabilities, and later on with substance abuse, anxiety, personality, affective and psychotic disorders, as well as with engagement in antisocial behaviors and school or work problems.

Furthermore, it has been shown that animals exposed to prenatal stress exhibit persisting modifications related to dopamine and glutamate transmission in limbic structures associated with dependence to alcohol and other substances. These alterations may later contribute to increase motivation to drink, to use large amounts of drugs of abuse or to relapse after periods of drug withdrawal. It was shown that after exposure to prenatal stress, male mice consumed more ethanol during alcohol reinforcement in adulthood.

In addition, it has been well documented that affective disorders are associated with alcohol dependence. A recent meta-analysis including 54 studies that together involved more than 40749 individuals, confirmed that the 5-HTTLPR polymorphism at the promoter of the serotonin transporter gene moderates the association between stress and depression, where the short allele is related with an increased risk for depression under stress ($p = 0.00002$). A strong association was detected when the stressful factor was childhood maltreatment ($p=0.00007$).

Childhood maltreatment, including neglect as well as physical and sexual abuse, is associated with developmental difficulties, low social competence and self-esteem, and it is an important risk factor for binge drinking in adolescence and alcohol dependence in adulthood. Childhood maltreatment may interact with factors such as variants of the monoamine oxidase-A and catechol-o-methyltransferase gene.

Adolescence is a critical period for initiation of alcohol intake, experimentation, and establishment of regular drinking patterns. Substance use at this age is considered a risk factor for the development of later alcohol and other drug-related problems, as well as for externalizing disorders such as antisocial personality disorder. Alcohol use initiation is affected by environmental factors such as ethanol availability, parental attitudes, and peer pressure. It

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has been reported that heavy drinking during adolescence can have a negative impact on brain development. Moreover, dopaminergic and GABAergic systems undergo important changes during adolescence, and they can be affected by alcohol intake. Dopamine is implicated in the rewarding effects of ethanol, and GABA in its sedating effects and development of tolerance.

The way an adult copes with environmental challenges is notably influenced by early life experiences and by the familial environment he or she had as an infant, which affects neurodevelopmental behavior.

While environmental factors tend to have a crucial role in drinking habits in adolescence, adultood may be characterized by a weaker effect of environment and a higher effect of genetic components.

It is probable that a complex set of gene–environment interactions determine the risk to alcohol dependence. Environmental factors that may affect this vulnerability appear at different stages from pregnancy to adulthood. These interactions are mediated by DNA methylation, histone modifications, protein complexes and non-protein-coding RNAs such as microRNAs.

Key words: Alcohol dependence, complex traits, gene-environment interaction, ethanol, alcoholism, epigenetics and alcohol, alcohol abuse in adolescence.

RESUMEN

La dependencia al alcohol es un problema mundial grave, que se asocia con mucho sufrimiento, problemas de salud mental y física, una elevada tasa de mortalidad y un costo social y familiar muy alto. Es por esto que las estrategias de prevención y el tratamiento de la enfermedad resultan cruciales. Se ha reportado que programas psicosociales y tratamientos farmacológicos son hasta cierto punto exitosos actualmente. Sin embargo, se requiere conocer más profundamente la etiología de la enfermedad. Por esta razón, es muy importante identificar los factores que se asocien con el incremento a la susceptibilidad en distintas poblaciones.

Se ha demostrado claramente a través de estudios en gemelos y de adopción, así como por investigaciones en animales, que tanto factores genéticos como ambientales son relevantes en la dependencia al alcohol. Se ha estimado que la heredabilidad de esta enfermedad se encuentra entre 40 y 60%, dependiendo de la muestra estudiada, lo cual indica que el ambiente y la genética tienen un peso similar en la susceptibilidad.

Muchas variantes genéticas que aumentan la susceptibilidad, en lugar de una sola, podrían coexistir en las personas más vulnerables a la dependencia. De manera similar, muchos factores ambientales parecen estar relacionados, los cuales, debido a su diversidad, intensidad y etapas de la vida en la que se presentan, no son exactamente iguales en diferentes personas con dependencia al etanol. La contribución ambiental podría relacionarse con cambios en la expresión de genes, lo cual involucra a la epigenética. Ésta se encarga del estudio de los procesos químicos, afectados por el ambiente, que pueden modificar la actividad de los genes sin cambiar la secuencia del ADN. En humanos, el proceso epigenético más estable es la unión de un grupo metilo (un átomo de carbón unido a tres átomos de hidrógeno) a las citosinas en el ADN. Otros mecanismos epigenéticos son modificaciones a proteínas nucleares llamadas histonas, proceso que modifica la manera en que se encuentra empaquetado el ADN. Por otra parte, ARNs que no codifican para proteína, como los microARNs, se han asociado al desarrollo de la dependencia al alcohol. Ciertos microARNs podrían funcionar como intermediarios epigenéticos, lo cual propiciaría que el etanol afectara procesos complejos y divergentes del neurodesarrollo, sumándose al efecto de la mutilación en el ADN y de la acetilación de histonas, entre otros procesos epigenéticos.

La mayoría de las investigaciones señalan que los genes importantes en la vulnerabilidad a la dependencia al alcohol incluyen algunos que codifican para proteínas del metabolismo del alcohol; de neurotransmisión de dopamina, GABA, serotonina, glutamato, opiodes endógenos y canabinoides; de transducción de señal en el sistema de recompensa mosolímbico; y de respuesta al estrés, entre otros.

Durante el embarazo, diversos factores no genéticos tienen un impacto importante en la vulnerabilidad a la dependencia al alcohol. Por ejemplo, debido a que el Sistema Nervioso se desarrolla a lo largo de toda la gestación y que el alcohol consumido por la madre puede llegar al feto a través de la barrera placentaria, el cerebro de un feto siempre es vulnerable al daño provocado por la exposición al etanol. Se ha demostrado que los hijos de mujeres alcohólicas no sólo pueden heredar variantes genéticas de riesgo sino que también pueden estar expuestos tempranamente a los efectos del alcohol consumido por sus madres. El consumo excesivo en el embarazo se ha asociado con retraso mental, epilepsia, déficit de atención/hiperactividad, problemas de aprendizaje, y más adelante con abuso de sutancias, ansiedad y trastornos afectivos, de personalidad y psicóticos, así como con conductas antisociales y problemas en la escuela o el trabajo.

Además, se ha demostrado que animales expuestos a estrés prenatal mostraban modificaciones persistentes relacionadas con la transmisión dopaminérgica y GABAérgica en estructuras límbicas que se relacionan con dependencia al alcohol y a otras drogas. Más adelante, estas alteraciones podrían contribuir a la motivación para beber, a utilizar mayores cantidades de alcohol u otras susancias de abuso o a la recaída después de periodos de abstinencia. Se encontró que ratones macho expuestos a estrés prenatal consumían más alcohol en la edad adulta.

La depresión y el estrés se han asociado fuertemente con la dependencia al alcohol. Con un metaanálisis reciente en el que se incluyeron datos de 54 estudios en los que en conjunto se habían reclutado 40 749 personas, se confirmó que el polimorfismo 5-HTTLPR del promotor del gen que codifica para el transportador de serotonina modera la asociación entre el estrés y la depresión y el alelo corto («S») se relaciona con un incremento en el riesgo a la depresión bajo el efecto del estrés ($p=0.00002$).

El maltrato en la infancia, incluidos abandono, descuido, abuso físico y sexual, se asocia con problemas en el desarrollo, baja autoestima y disminución en las aptitudes y es un factor de riesgo importante para el consumo excesivo de alcohol en la adolescencia y dependencia a esta sustancia en la edad adulta. El maltrato infantil podría interactuar con factores como ciertas variantes de los genes de la monoamino oxidasa –A y de la catecol-o-metiltransferasa.

La adolescencia es una etapa importante respecto al inicio de ingesta de alcohol, experimentación y establecimiento de patrones de consumo regulares. El uso de sustancias en este período se considera un factor de riesgo para el desarrollo posterior de problemas relacionados con el abuso en el consumo de alcohol y de otras sustancias, así como con trastornos externalizados, incluida la personalidad antisocial. Por otro lado, el inicio del consumo a esta edad está afectado por factores ambientales como la disponibilidad de alcohol, las actitudes que los padres adoptan y la presión de los compañeros. El consumo excesivo de etanol durante la adolescencia puede tener un impacto negativo en el desarrollo cerebral. Además, los sistemas dopaminérgico y GABAérgico presentan cambios importantes durante la adolescencia y pueden ser afectados por el consumo del alcohol. La dopamina está implicada en los efectos de recompensa provocados por la ingesta de la sustancia y el GABA en sus efectos sedantes y en el desarrollo de tolerancia.

Por otra parte, aunque los factores ambientales tienden a ser muy relevantes en los hábitos de consumo en la adolescencia, en la edad adulta parece haber un efecto ambiental menor, pero un mayor peso de los factores genéticos.

Los factores genéticos que aumentan la susceptibilidad a la dependencia al alcohol tienen distinto peso dependiendo del grupo de edad de las personas. Los componentes ambientales que aumentan la vulnerabilidad a esta enfermedad se presentan en diferentes etapas de la vida desde el embarazo hasta la edad adulta. A lo largo de la vida se van acumulando factores de riesgo o protección. Las interacciones genético-ambientales están mediadas por la metilación del ADN, modificaciones covalentes en las proteínas histonas, complejos proteicos y por ARNs que no codifican para proteína, como los microARNs.

Palabras clave: Dependencia al alcohol, trastornos complejos, interacción gen-ambiente, etanol, alcoholismo, epigenética y alcohol, abuso en el consumo de alcohol en la adolescencia.

INTRODUCTION

Ethanol is one of the most socially accepted addictive drugs worldwide. It tends to be beneficial for many people if used moderately, but abuse and dependence to it represent a major global problem, associated with lower quality of physical and mental health, higher mortality and an enormous familial and social cost.[1] Prevention strategies and treatment of these conditions are therefore crucial. Psychosocial programs and pharmacological treatments for alcohol dependence are successful to some extent, but a better understanding of the etiology of this chronic disease is needed. For this purpose, the identification of specific associated factors in different populations is of great significance.

Family studies indicate that the risk for alcohol dependence is 4 to 10-fold higher in offspring of an alcoholic parent. It has been clearly demonstrated by twin and adoption studies and supported by animal models that both genetic and environmental components play a substantial role in alcohol dependence. A meta-analysis considering nearly 10 000 twin pairs showed that heritability is about 50%; similarly, different studies indicate it is 40 to 60%, which is still far from the expected 100% in a purely genetic disorder. Genetic and non-genetic factors that are in fact associated with alcohol dependence have been identified in different populations.[2]

Several coexisting genetic variants in each affected individual, rather than a single variant (allele) transmitted in a Mendelian manner, may be the rule in the susceptibility to alcohol dependence. In addition, genetic heterogeneity may be present, so unrelated affected subjects could bear a different (or partially different) set of vulnerability alleles. Moreover, incomplete penetrance is expected due to the contribution of environmental and epigenetic factors and the probably moderate effect of susceptibility alleles. Incomplete penetrance is a state in which genetically vulnerable individuals may never develop alcohol dependence. This suggests that a single DNA variant that is associated with the disease may neither be absolutely necessary nor sufficient to strikingly increase the risk for the disease in absence of other susceptibility factors, at least in most cases. Similarly, many environmental factors can increase susceptibility, and because of their diversity, intensity and stage of life in which they are present, they do not have to be the same in every affected person.

Environmental contribution may be linked to epigenetics, which refers to chemical processes that can modify gene activity without changing the sequence of DNA. In humans, the most stable epigenetic process is the union of a methyl group (one carbon atom surrounded by three hydrogen atoms) to cytosines in DNA. Methylation may be subject to dynamic regulation through embryogenesis and postnatal life.

Other epigenetic mechanisms are modifications to nuclear proteins called histones, which define the way DNA is packed into groups of little repeating units called nucleosomes. Each nucleosome contains a core, where the DNA is placed around a histone octamer (figure 1), as well as an area of relatively relaxed DNA called the linker DNA. Nucleosome cores may block DNA-binding factors, so their exact position in the sequence of DNA is important to define chromatin packing and gene regulation. Regulation occurs at different levels, such as transcription, that describe the production of RNA based on the sequence of the DNA template, replication, when new copies of DNA are made, and recombination, when stretches of DNA are exchanged between homologous chromosomes during meiosis, and DNA repair.[3]

It has been suggested that besides transcriptional machinery, epigenetic signals are needed to establish, maintain and reverse gene expression states required by the cell to retain information about past events, including changes in the environment or developmental cues.[4] For example, epigenetic modifications may have a crucial role in neuronal gene expression response to this kind of cues.[5] An accurate gene transcription control in response to environmental signals is needed for the development and function of the CNS, a control that is mediated also by epigenetic factors. Furthermore, there are RNA-based regulatory networks including transfer, ribosomal, micro, small nucleolar, and long non-protein-coding RNAs that are involved in most cellular processes. It has been suggested that microRNAs could work as epigenetic intermediaries allowing ethanol to affect complex and divergent developmental processes.

Given that certain periods in life tend to be associated with specific events related with the onset of alcohol

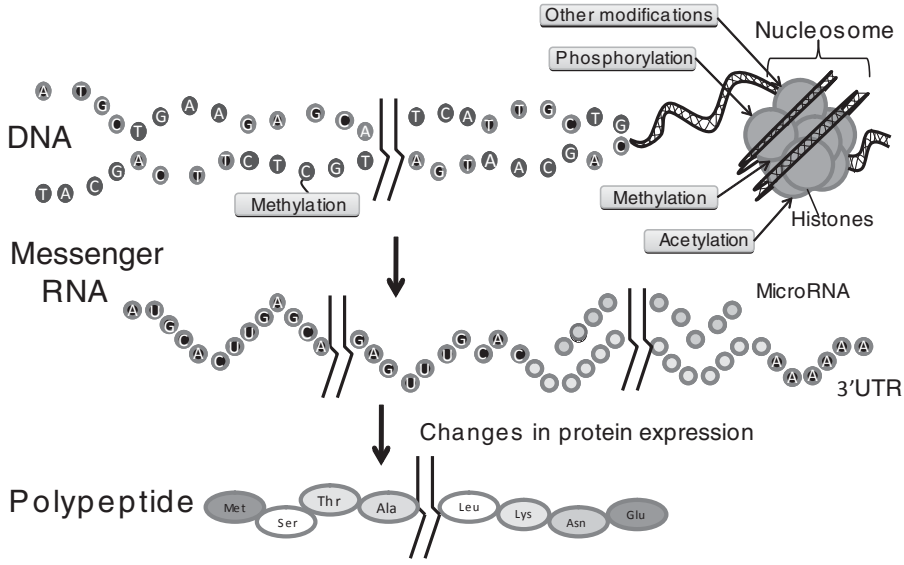

Figure 1. Epigenetic factors such as DNA methylation and histone modification, as well as microRNAs affect gene expression and protein production.

dependence, in this article we will discuss the evidence on the impact that genetics and environment exert to affect susceptibility to alcohol dependence at different moments in life, including pregnancy and childhood, adolescence and adulthood, and how epigenetics could affect the phenotype. Other studies have taken into consideration different stages in life. Subtypes of alcoholism, for example, have been suggested according to age at onset of the disease, among other characteristics. Cloninger proposed that type 1 alcoholism was characterized by anxious personality traits and a fast development of tolerance and dependence on the anti-anxiety effects of ethanol, associated with loss of control, difficulty terminating binge drinking, guilt, and organic complications. Conversely, type 2 alcoholism was described as antisocial personality traits and continuous seeking of ethanol for its euphoriant effects, associated with an early inability to abstain entirely, fighting and arrests.[6]

PREGNANCY AND CHILDHOOD

According to most estimates, although chimpanzee and human lineages diverged about 6 million years ago, their genetic differences are only 10 to 12 times greater than those between two unrelated people. This is possible because DNA is passed virtually unchanged to new generations (if we think about the entire length of the genome). High fidelity copies of DNA allow maintaining genetic information over time and in some cases avoid mutations that may promote human diseases such as alcohol dependence.[7]

Despite DNA copying fidelity, sometimes fragments of the sequence are altered. Substitutions and short to long deletions or insertions, inversions or translocations of DNA fragments may occur spontaneously through random errors in copying of DNA, or may be induced by chemicals or radiation. In addition, errors in RNA or protein production can happen. However, a mutation should be present in sex cells in order to be passed down to offspring and only in some instances will it affect characteristics in the next generations. Some DNA changes become frequent at a population level, so they are no longer considered mutations but polymorphisms. Within the approximately 0.01% of the DNA sequence that is not shared between two unrelated individuals, there must be several alleles that could make the first person more or less vulnerable to alcohol dependence than the other. Among those genetic variants, most reasearch points to those related with alcohol metabolism, neurotransmission of dopamine, GABA, serotonin, glutamate, endogenous opiods and cannabinoids, signal transduction within the mesolimbic dopamine reward system, and stress response system among other alleles.[8]

When a spermatozoa and an egg cell fuse during fertilization, a zygote is formed with a unique version of paired DNA sequence. This new amalgam of DNA is the product of random recombination through breaking and rejoining of previously independent grandmaternal and grandpaternal DNA transmitted by the father, and a similar contribution of DNA from maternal grandparents transmitted by the mother. Thus, the new individual will share a random 50% of nuclear DNA sequence with each parent (a complete haploid version of the human genome). Given that both parents contribute with a haploid copy of the genome, the new individual is diploid.

After fertilization, the zygote begins a process of cellular division in which additional identical copies of its

DNA are generated for each new cell. This process allows the formation of an embryo, and eventually an individual with trillions of specialized cells with the same genetic information but exposed to specific regulation at the transcription, post-transcriptional, translational and post-translational level.

It would be easier to predict possible consequences in a newborn if alcohol dependence were determined by a decisive effect of a single gene. However, there may be several risk and protective genetic variants present in the ancestors of a baby, some of which may have been transmitted and some others which may have been lost in this particular lineage.

During pregnancy, there are several non-genetic factors that may have an important impact on vulnerability to alcohol dependence. Children born to alcoholic mothers may inherit genetic susceptibility variants but at the same time they may be exposed to early effects of ethanol. Given that the Central Nervous System is developing throughout the entire pregnancy and alcohol consumed by the mother can reach the fetus through the placental barrier, the brain of a baby is always vulnerable to harm caused by alcohol exposure. Heavy alcohol exposure during gestation has been associated with mental retardation, epilepsy, attention deficit / hyperactivity disorder, and learning disabilities, and later on with substance abuse, anxiety, personality, affective and psychotic disorders, as well as with engagement in antisocial behaviors and school or work problems.[9]

It has been shown that alcohol may interfere with brain cell migration and organization (first trimester), and it is related with fetal alcohol syndrome (mostly during the second trimester, especially at weeks 10 to 20). Several brain regions, some of them belonging to the limbic system, such as hippocampus, amygdala and hypothalamus, could be affected, and are associated with emotion, social behavior, and aggression.[10] It has been shown that prenatal alcohol exposure increases the activity of the hypothalamic-pituitary-adrenal axis and results in a dysregulation of it throughout life, which may be common in depression and anxiety disorders.[11] In rats, fetal exposure to ethyl alcohol was associated with an infantile affinity for alcohol[12] and an enhanced behavioral response to ethanol's odor, especially in adolescence, which could contribute to progressive acceptance of this drug later on.[13]

Also recently, female rhesus monkeys were analyzed. Some of them were exposed to alcohol in different gestational stages or during the whole pregnancy; others were only exposed to an isocaloric solution (control group). Serotonin 5-HTTLPR-polymorphism was genotyped and levels of primary serotonin and dopamine metabolites in cerebro-spinal fluid were determined. Carriers of the short allele tended to have reduced 5-hydroxyindoleacetic acid (5-HIAA) in early- and middle-to-late gestation alcohol-exposed monkeys and reduced homovanillic acid (HVA) at baseline.[14]

Alterations in glutamate release have been suggested in an animal model of prenatal exposure to ethanol. These changes may be associated with a decreased efficiency of excitatory synaptic activity in the hippocampus during postnatal life.[15]

Prenatal alcohol exposure could permanently disrupt other neurotransmitter systems. Furthermore, it has been shown that people with alcohol dependence may have altered zinc metabolism and alcohol use during pregnancy has been associated with reduced zinc levels in maternal plasma and fetal core blood, which could contribute to the adverse effects alcohol exerts on development. According to animal studies, a marginal zinc deficiency could increase teratogenic effects of alcohol while a zinc supplementation could prevent prenatal ethanol's adverse effects on neurodevelopment.[16]

In addition, prenatal stress may also alter neuro-development, resulting in hyperresponsiveness to stress, novelty, and stimulant drugs. It has been shown that animals exposed to prenatal stress exhibit persisting modifications related to dopamine and glutamate transmission in limbic structures, which are associated with dependence to alcohol and other substances. These alterations may contribute to a higher motivation to drink, to use large amounts of drugs of abuse or to relapse after periods of drug withdrawal. After exposure to prenatal stress, male mice consumed more ethanol during alcohol reinforcement once they were adults.[17] In other preclinical investigations, it was shown that central corticotropin-releasing factor (CRF) was persistently hyperactive after exposure to stress early in life. Increased reactivity to stress in adulthood was also reported. CRF is the central coordinator of the endocrinologic, autonomic, immunologic, and behavioral responses to stress, which may be related with a higher susceptibility to anxiety and depression with new exposures to stress.[18]

Gene-environment interactions have been proposed. Adult carriers of the short allele of the 5-HTTLPR polymorphism tended to have increased levels of depression in presence of high levels of concurrent stress or if they had a history of childhood neglect or abuse.[19] In addition, it has been reported that young children who carry the short allele and who have an uncertain attachment relationship to their mother may be behaviorally inhibited, which is also a risk factor for affective disorders. Furthermore, it has been well documented that affective disorders are associated with alcohol dependence. A recent meta-analysis including 54 studies that originally involved more than 40 749 individuals confirmed that 5-HTTLPR polymorphism moderates the association between stress and depression, where the short allele is related with an increased risk for depression under stress ($p=0.00002$). The analysis for specific kind of stressors showed strong evidence for association between the short allele and increased stress sensitivity in a childhood maltreatment ($p=0.00007$) and a specific medical condition ($p=0.0004$) group, respectively.[20]

Regarding microRNAs, one study revealed that a high dose of ethanol could suppress the expression of four microRNAs: miR21, miR335, miR9, and miR153. However, a moderate ethanol dose could only suppress miR335 expression. Although supression of miR21 could result in apoptosis, miR335 could have an antagonistic effect that should be further analyzed.[21]

Childhood maltreatment, including neglect, as well as physical and sexual abuse, is associated with developmental difficulties, low social competence and self-esteem, and it is an important risk factor for binge drinking in adolescence and alcohol dependence in adulthood. A report from the Mexican National Comorbidity Survey (M-NCS) showed that childhood adversities have an effect on lifetime risk of substance use disorders. In this case, parental criminal behavior and substance use, family violence, physical abuse, and neglect predicted substance use disorders, while other adversities such as parental mental illness, sexual abuse, and death of a parent showed moderate effects in the onset of substance use disorders.[22]

Likewise, childhood maltreatment may interact with a variant of the monoamine oxidase A (MAOA) gene to predict antisocial behavior which is often associated with alcohol dependence. In addition, an interaction of the serotonin transporter 5-HTTLPR polymorphism predicts alcohol abuse in nonhuman primates and with depression in humans. Moreover, the Met158 variant in the catechol-O-methyltransferase (COMT) gene may confer risk and resilience to alcohol dependence in different environments.[8]

Personality traits observed in childhood may be associated with a higher risk to alcohol dependence. It has been shown that children with high novelty-seeking and low harm avoidance may be at greater risk for early-onset alcohol abuse.[23]

Many of the risk factors for early drinking emerge during the first decade of life. From pregnancy to childhood, the brain undergoes an exceptional growth and it is modified in its structure, organization, and function. By age 10, many adaptive systems have assembled and exhibit certain stability. Children arrive at adolescence with the particular experiences, achievements and failures they accumulated earlier, with genetic and epigenetic elements that may affect their vulnerability to alcohol dependence later in life, as well as knowledge about alcohol use and its effects through their exposure to different kinds of advertisements, but still parents tend to be the main source of children's exposure to alcohol use.[24]

ADOLESCENCE

Adolescence is a critical period for initiation of alcohol intake, experimentation, and establishment of regular patterns of drinking. Substance use at this age is considered a risk factor for the development of later alcohol and other drug-related problems, as well as for externalizing disorders such as antisocial personality disorder.

Indeed, frequent and heavy drinking at this stage has been shown to increase the risk for alcoholism,[25] especially if age at first drink is early in life. Furthermore, it is associated with problems such as obesity, high blood pressure, headaches, suicide, deficit in concentration, memory, and learning, as well as with traffic accidents and unsafe driving, violent behaviors, unwanted and unprotected sexual activity, and poor academic attainment.[26]

If alcohol use starts before the age of 15 years, there is a 4-fold increased risk for lifetime alcoholism as contrasted with initiation at 21 years.

In average, the age of the first drink is 11 years for boys and 13 years for girls in the United States. While the age of first drink was 16 years for boys and 18 for girls in urban Mexico about a decade ago,[27] currently there is a general tendency to begin drinking earlier, particularly in girls, and 3.6% of boys and 2.1% of girls who are 12 to 17 years old may already have a diagnosis of abuse or dependence to alcohol.[28] Binge drinking (defined as ≥5 drinks/occasion in any 2-week period) is common in teenagers; according to some reports, it may be related with their idea of improving social skills.

Drinking initiation is affected by environmental factors such as alcohol availability, parental attitudes including monitoring, rules, parent-child attachment, alcohol use, and tolerance to alcohol use, attitudes of other relatives, and peer pressure at school and community. It has been suggested that a younger age of first drink may be predicted by the genotype of serotonergic 5-HTTLPR polymorphism.[29]

Children and adolescents learn about many aspects regarding alcohol use, such as the way it produces changes in cognition, feelings, and behavior, its position in social

Table 1. Risk factors that may be associated with alcohol dependence

Shared environment • Prenatal alcohol exposure and stress. • Attachment, neglect, violence, abuse. • Parental criminal behavior, mental illness, substance use/alcohol dependence, tolerance on alcohol use. • Religiosity, cultural aspects.	Genetic variants related with: • Alcohol metabolism, neurotransmission, signal transduction, stress response, other processes. • Psychiatric comorbidity, personality traits.
Non-shared environment • Abuse, stressful events/disease. • Peer pressure, alcohol availability, patterns of drinking of friends. • Heavy drinking. • Marital status, changes in religiosity. • Use of leisure time. • Cultural aspects. Lack of social restriction for excessive drinking.	Gene regulation and RNA editing • Transcription factors. • Other protein complexes. • DNA methylation. • Histone modification. • Nucleosome position. • Non-protein-coding RNAs

relationships, the kind of people that drink ethanol, and possible reasons for using it, so they develop expectancies about its use.[24]

It has been reported that heavy drinking during adolescence may have a negative impact on brain development, especially in the hippocampus. Permanent modifications in adult brain caused by binge drinking have been described in animals.[30]

Dopaminergic and GABAergic systems undergo important changes during adolescence, and they can be affected by alcohol intake. Dopamine is implicated in the rewarding effects of ethanol, and GABA in its sedating effects and development of tolerance. Low response to the sedating effects of alcohol is associated with a 4-fold increase in risk for future alcoholism.[31] Low reponders who start drinking very early may be at greater risk.

Hyperactivity and conduct problems in 8 year-old boys are associated with frequent drunkness 10 years later.[32] In addition, antisocial behavior has been linked to regular alcohol use in early adolescence and alcoholism later in life.[25]

It has been described that drinking to alleviate negative feelings is associated with heavy drinking and alcohol related problems in adolescence. This was confirmed in a study of 282 Dutch teenagers, where the A1 allele of the Taq1A polymorphism at the dopamine receptor DRD2 gene was associated with drinking to forget negative feelings, binge drinking, and alcohol related problems.[33]

ADULTHOOD

The way an adult copes with environmental challenges is notably influenced by early life experiences and by the familial environment he or she had as an infant (table 1).

As mentioned before, ethanol exposure during pregnancy, childhood, and adolescence increases the risk for alcohol use disorders in adulthood. Animal models support a similar situation. Rodents who were exposed to poor maternal contact early in life tended to show neurobiological modifications that persist into adulthood, including expression-related alterations in the hippocampus.[34] The combination of epigenetic effects and drinking at a young age may account for part of the increase in vulnerability to addiction. Relevant environmental factors include marital status and religiosity; being married and some religiosity variables tend to be protective factors against alcohol dependence.[35]

Nevertheless, while environmental factors tend to play a crucial role in drinking habits in adolescence, adultood may be characterized by a weaker effect of environmental components and a higher effect of genetic factors.[7] Genetics may have an effect on quantity of alcohol consumed in a typical occasion, frequency of intake, frequency of intoxication, alcohol metabolism (time to reach maximum ethanol or acetaldehyde concentration in blood, elimination rate), tolerance, abstinence, and age at onset of dependence.

Alcohol metabolism genes

Ethanol is mainly oxidized either by alcohol dehydrogenase (ADH), cytochrome P4502E1 (CYP2E1), or catalase, a reaction that produces acetaldehyde, which is oxidized by the aldehyde dehydrogenase 2 (ALDH2) with the production of acetate. It has been shown that several polymorphisms at the ADH and ALDH2 genes affect the oxidizing capacity of the enzymes they encode. The frequency of each genetic variant (allele) changes across populations. Several ADH and ALDH genetic variants have been associated with alcohol intake, alcohol-related tissue damage, and alcohol dependence. On the other hand, some variants are protective against dependence to ethanol. High-activity ADH variants as well as low activity (or essentially inactive) ALDH*2 tend to be protective; they are associated with higher accumulation of acetaldehyde, which is probably the main cause of the intense and unpleasant physiological and psychological reaction experienced by carriers of protective alleles. ADH1B*2 allele is very common in Asian populations.[36] In contrast, in a Mexican sample from the Huichol Indian population, the ADH1B*2 frequency was 0% and in a Mestizo Mexican sample the frequency for the same allele was 3.4%.[37] Moreover, ADH1B*3 allele may only be present in African descent and some Native American populations. This genetic variant has also been associated with rapid alcohol oxidation, leading to a temporary increase in acetaldehyde concentration. As happens with other protective gene variants, African Americans who are carriers of this allele are less likely to have a family history of alcohol dependence and tend to have an unpleasant response to alcohol.[38]

With respect to ADH1C, a higher frequency of the protective allele ADH1C*1 was found in a North African (Moroccan) population when compared to a Basque population.[39]

Regarding the ALDH protective alleles, the ALDH2*2 variant causes an amino acid change from glutamate to lysine (when compared to the ancestral ALDH2*1 allele), a change that drastically reduces enzymatic activity. ALDH2*2 allele is associated with a slower acetaldehyde oxidation rate, regardless of being present in both maternal and paternal chromosomes (in homozygotes for *2 allele) or when only one copy is carried (heterozygotes *1*2). ALDH2*2 was extremely rare in the Mexican Huichol sample (frequency of 0.5%), but more common (16%) in a Mestizo sample from the same country.[37]

Acetaldehyde is a genotoxic compound that reacts with DNA to form adducts that eventually block DNA repair mechanisms. In addition, alcohol intake induces the expression of CYP2E1, which causes an increase in reactive oxygen species and DNA damage. There are some studies

that support the notion that chronic alcohol exposure is an important risk factor for cancer at different parts of the body, including oral cavity, pharynx, larynx, esophagus, and liver.[36]

In Mexico, individuals of Otomi Indian ancestry were compared according to the presence or absence of alcoholism. Differences in allelic frequencies between alcoholics and non-alcoholics were identified at the CYP2E1/TaqI polymorphism, where homozygosity for the A2 allele (genotype A2A2) was more common in nonalcoholics. The frequency of the A1 allele was significantly more common in alcoholics.[40] Futhermore, the Huichol Indian sample showed the highest worldwide reported frequency for other variant, the CYP2E1*C2.[37]

Genes related with neurotransmission and stress response

As mentioned earlier, alcohol dependence is associated with the mesolimbic reward system, and several dopaminergic genes such as the dopamine transporter (SLC6A3) and the DRD2 and DRD4 receptor genes have been associated with the disease.

Furthermore, GABAergic genes such as the GABA(A) α-6 subunit gene have been frequently associated with variation in sensitivity to ethanol. The GABRA2 gene has been associated with alcohol dependence. More than 1900 individuals that belong to families with several alcoholic members were evaluated from a sample of the Collaborative Study of the Genetics of Alcoholism (COGA). A high risk genotype was correlated with divorce, and negatively correlated with marriage.[41]

Regarding the glutamatergic system, variants of the glutamatergic N-methyl-D-aspartate receptor (NMDAR) have been associated with alcoholism.[42]

Moreover, the lower transcribing short allele of the 5-HTTLPR polymorphism may contribute to the risk for alcohol dependence in adulthood, with the strongest effect in individuals with more severity of the disease, antisocial behavior, and suicide attempts.[43]

Moreover, the cannabinoid system is part of the mesocortical reward pathway. The pathological effects of chronic ethanol intake may be mediated by this system.[44] The «AA» genotype of a silent polymorphism (Thr453Thr) in the CB1 cannabinoid receptor gene (CNR1) has been recently associated with alcohol withdrawal delirium ($p=0.031$) in a Caucassian sample.[45] After initial reports of association between the CNR1 gene and substance dependence, a sample of European Americans was analyzed. Substance dependence (including alcohol dependence) was associated with the CNR1 gene, with the strongest effect shown for markers rs6454674 and rs806368.[46]

Molecular changes in the endogenous opioid system may be related with neuroadaptations that are key elements for progression to dependence.[5] Patients with alcohol dependence who carry the Asp40 variant in exon 1 of the μ-opioid gene (OPRM1) are more likely to respond to naltrexone, an opioid receptor antagonist with known efficacy.[47]

In addition, brain stress and fear systems become activated in later stages of alcohol dependence and their activation is an important influence in susceptibility to relapse. The corticotropin-releasing hormone (CRH) may be related with changes in the stress response system after repeated administration of ethanol. The rs10055255 polymorphism in the corticotrophin-releasing hormone binding protein (CRH-BP) gene may affect stress-induced craving and may be related with a greater affective response to stress.[48] Recently, genetic inactivation of neurokinin 1 (NK1) receptors was shown to block alcohol reward in rodents and to decrease alcohol craving in humans, while NK1 antagonism decreases alcohol craving in humans.[49]

Gene expression and epigenetics

Ethanol intake alters gene expression patterns and it is related with cellular and molecular adaptation thay may explain the development and chronicity of alcohol dependence. Motivational behavior, reward and learning, as well as adaptations in the signaling pathways are involved in the etiology of alcohol dependence.

Transcription factors such as CREB may be relevant in the etiology of this disease. In addition, epigenetics may underlie the neuroplasticity commonly observed in dependence. External factors such as alcohol itself could induce alterations in the epigenome, thus affecting gene regulation. In addition, it has been suggested that chromatin remodeling could be related with dependence to ethanol.[50] In addition, the epigenetic regulation of the dopamine transporter gene promoter may be altered in patients with alcohol withdrawal. Hypermethylation of this promoter may be involved in dopaminergic transmission and a decreased alcohol craving.[51] Likewise, differences in methylation of the pro-opiomelanocortin gene (POMC) promoter were identified when samples of alcohol-dependent individuals and healthy controls were compared, and a specific association with craving was detected. This finding also suggests that epigenetic changes possibly due to alcohol intake may contribute to alcohol craving.[52]

The glutamatergic system has been linked to alcohol dependence at the epigenetic level. When part of the NR2B receptor gene promoter was analyzed, the severity of alcohol consumption was negatively correlated with the methylation of a defined region within the promoter, which may explain the impact of alcohol intake patterns on withdrawal symptoms.[53]

Furthermore, changes in the concentration of the amino acid homocystein may influence DNA-methylation of specific genetic areas affecting the production of proteins that are possibly relevant for development and maintenance

of alcohol dependence.[54] Altogether, these data support the notion that epigenetics plays an important role in alcohol dependence.

DISCUSSION

The involvement of genetic and environmental factors in the etiology of alcohol dependence has been established through different lines of research. Preventive strategies should take into consideration that these two branches of risk components are important at different stages in life, even prenatally, and that family involvement is relevant.

Regulatory elements such as transcription factors, DNA methylation, histone modifications, protein complexes, and non-protein-coding RNAs such as microRNAs may have a direct or indirect effect on gene expression and RNA editing. MicroRNAs should be further investigated, as they may mediate gene-environment interactions increasing the risk to alcohol dependece.

The identification of genes involved in susceptibility to ethanol dependence should not be regarded exclusively as a possible means of discrimination, because genes are not determining elements but rather a source of differences in susceptibility. Genetic studies could help identify personalized treatments based on pharmacogenetic and pharmacogenomic studies; they could also help to promote prevention strategies and early interventions in families thay may have a higher genetic risk, which may favor a more positive environment during pregnancy and later stages of life, possibly leading to better family functioning, less distant relationships between parents and offspring, closer parental monitoring, less violence and abuse, and even early treatment for improving mental health in parents and progeny. Young individuals that are vulnerable to drug abuse and later to dependence should be aware that change toward a more positive environment may be helpful and genetic variants explain only a part of the variation in susceptibility to dependence. However, there are known protective genetic factors that are strongly associated with the absence of alcohol abuse and dependence even in risky environments.

The most studied genes are related with alcohol metabolism and the frequency of allelic variants tends to differ depending on ethnicity. For this reason, alleles that are protective to Asian populations, for example, may be rare in other groups, where other putative protective variants may be common. Given that there is ethnic heterogeneity, specific studies should be performed in different populations.

Other relevant genes may be related with neurotransmission and stress response, but it is possible that a different, less studied group of genes increases or modifies susceptibility to alcohol dependence, either directly or through comorbid conditions.

A strong positive family history of alcohol dependence represents a high risk for the disease, which is related with shared genetic and environmental factors. Childhood maltreatment also increases the risk for alcohol dependence, while in adolescence the added contribution of non-shared environmental factors tends to be crucial. Many elements influence juvenile alcohol intake, and each variable plays a partial role in consumption behavior. In adulthood, genetic effects tend to be stronger, but earlier negative environmental factors may still have an important impact on susceptibility to dependence.

Future research should take into consideration genetic heterogeneity, possible ethnic differences in frequency of each allele, environmental variables in different stages of life, as well as gene-environment interaction mediated by DNA methylation, histone modifications, protein complexes and non-protein-coding RNAs such as microRNAs. Furthermore, it is now possible to carry out genome-wide DNA, expression, and epigenetic analyses in well characterized samples.

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