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Functional Polymorphisms of Alcohol Metabolism Genes and their Protection Against Alcoholism via Gene Therapy: A Systematic Review

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Abstract

Around 45% of East Asians are unable to fully metabolize ethanol due to functional polymorphisms of alcohol metabolism genes, specifically alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). These individuals display high levels of toxic blood acetaldehyde when consuming alcohol, causing symptoms such as tachycardia, vomiting, and flushing. The studies included in this review examine how functional polymorphisms of ADH and ALDH protect against alcoholism in addition to how polymorphisms can be utilized as protection against alcoholism via gene therapy. The studies included found that carriers of the ADH and ALDH polymorphisms were 66 to 99% genetically protected against alcoholism. Through the use of gene pharmacology involving ADH and ALDH mimicry, gene therapy was seen to reduce ethanol intake in several animal models, furthering the development of new treatments for alcoholism.

Keywords: aldehyde dehydrogenase, alcohol dehydrogenase, Asian, flush, polymorphism, gene therapy, alcoholism

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INTRODUCTION

Many individuals around the world consume alcohol in safe levels. However, a significant number of individuals dangerously increase their alcohol intake in a way that is susceptible to addiction and alcohol use disorder (AUD) (Enoch, 2014). AUD is a type of substance use disorder (SUD) and is a moderately heritable chronic medical illness that is very common, affecting an estimated 15 million people in the United States (National Institute on Alcohol Abuse and Alcoholism, 2015). SUDs "are characterized by clinically significant impairments in health, social function, and impaired control over substance use and are precisely diagnosed through assessing cognitive, behavioral, and psychological symptoms" (U.S. Department of Health and Human Services, 2016, pg. 6). According to the Substance Abuse and Mental Health Services Administration (2017), 20.1 million people (6.2% of the US population) met the diagnostic criteria for SUD in 2016, but only 3.8 million individuals (18.9% of those with SUD) received any type of treatment. In the case of SUD related to alcohol use, Hasin, Stinson, Ogburn, and Grant (2007) noted that the lifetime prevalence of AUD in the United States is 30% and has continued to rise each year.

When an individual becomes addicted to alcohol use, they experience the most severe form of AUD. Addiction in the context of AUD is associated with compulsive and uncontrolled use of alcohol (U. S. Department of Health and Human Services, 2016). The process of developing an addiction to alcohol involves a number of functional genetic factors in addition to environmental factors. According to Goldman, Oroszi, and Ducci

(2005), the heritability of alcoholism is about 50%. However, only two genes have been verified as "addiction genes" in the human body within the context of alcoholism: mitochondrial aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) (Goldman et al., 2005; Higuchi, Matsushita, Murayama, Takagi, & Hayashida, 1995; Luczak, Glatt, & Wall, 2006). Both genes are polymorphic in humans, meaning that more than one allele can occupy each gene's locus along a chromosome within a population of humans. For example, two forms of ALDH2 exist: ALDH2*2 and ALDH2*1. ALDH2*2 encodes the inactive form of the ALDH2 enzyme while ALDH2*1 encodes the active, more frequent form of the ALDH2 enzyme. The difference in alleles located at each gene's locus results from changes in single nucleotides, which alter the activity of each enzyme (Pautassi, Camarini, Quadros, Miczek, & Israel, 2010). This is known as a single-nucleotide polymorphism (SNP) (C. Chen, Lu, Y. Chen, Wang, Chang, Li, & Yin, 1999). Because polymorphisms of each gene can alter the gene's final functioning capacity, all genetic variants of ALDH and ADH are both referred to as functional polymorphisms.

The body's way of metabolizing alcohol typically includes breaking down ethanol within the liver via the ADH enzyme into acetaldehyde while then utilizing the ALDH enzyme to further metabolize acetaldehyde into nontoxic acetate (Enoch, 2014; Rivera-Meza, Quintanilla, Tampier, Mura, Sapag, & Israel, 2010). Those who have a polymorphism in the ADH and ALDH enzymes are unable to properly metabolize alcohol, leading to the accumulation of acetaldehyde, which is mutagenic and carcinogenic to the body. Because the body is unable to fully metabolize ethanol, the consumption of alcohol causes a buildup of acetaldehyde in the body which then causes

symptoms of flushing, hypotension, nausea, headache, vomiting, and tachycardia, ultimately discouraging further alcohol intake (Chen et al., 1999; Goldman et al., 2005; Pautassi et al., 2010). This response to alcohol consumption is known as the *flushing response*, *Asian glow*, and *Asian flush* (Higuchi et al., 1995). Individuals who possess functional polymorphic alleles of ADH and ALDH are said to have a genetic protection against alcohol abuse and alcoholism. In fact, individuals who are heterozygous ALDH2*2/ALDH2*1 carriers are protected against alcohol abuse and alcoholism by 66 to 75% while homozygous ALDH2*2/ALDH2*2 carriers are nearly 100% protected (Ocaranza, Quintanilla, Tampier, Karahanian, Sapag, & Israel, 2008). According to Enoch (2014), approximately 45% of East Asians (Japanese, Chinese, Koreans, Thais) are carriers of the ALDH2*2 allele. More specifically, approximately 31% of Chinese, 45% of Japanese, 29% of Koreans, and 10% of Thais carry at least one of the variant ALDH2*2 allele (Luczak et al., 2006). However, the same allele is rare in Western and Central European Whites; although it remains moderately prevalent in Russian and Jewish Whites.

To understand the interaction between functional polymorphisms of the alcohol metabolism genes and their protection against alcoholism, it is important to first understand the variations and roles of the major enzymes of alcohol metabolism, ADH and ALDH. Four genes are responsible for coding the four forms of ADH that are involved in alcohol metabolism (Chen et al., 1999). The four forms of ADH can be split between class I (ADH1-3) and class II (ADH4) enzymes. At the ADH2 locus, three allelic variants are possible: ADH2*1, ADH2*2, and ADH2*3; at the ADH3 locus, two allelic variants are possible: ADH3*1 and ADH3*2. These variations in alleles that code

for ADH can dramatically impact the enzyme's rate of activity within the cell. The same is true of the allelic variants of the two forms of ALDH: cytosolic ALDH1 and mitochondrial ALDH2. The ALDH2 locus contains two allelic variations: ALDH2*1 and ALDH2*2.

According to Chen et al. (1999), the alleles ADH2*2 and ADH3*1 code for different forms of the high-activity ADH enzyme along with the ALDH2*2 allele that codes for the low-activity form of ALDH2. The frequency of these three allelic variants have been documented to be significantly low in alcoholics when compared to a general population of East Asians. Chen and colleagues (1999) tested the hypothesis that the allelic variants ADH2*2, ADH3*1, and ALDH2*2 provided substantial protection against alcoholism through either faster production or slower removal of acetaldehyde among 885 alcoholics and controls of Han Chinese descent in Taiwan. ADH2 and ADH3 haplotype frequencies were combined with ALDH2 genotypes and then compared between the participants diagnosed with alcohol dependence (alcoholic group, n = 340; 315 males and 25 females; mean age = 40) and a control group (n = 545; mean age = 20) comprised of male medical, dental, and pharmacy students that were either nondrinkers or occasional light drinkers. Genomic DNA was extracted from subjects' leukocytes and then labeled via polymerase chain reactions (PCR) by their SNP sites to determine each subject's differences in genotypes and alleles associated with alcohol metabolism.

Chen et al. (1999) found that genotype and allele distributions of the alcohol metabolism genes were not significantly different among the Han Chinese alcoholics group; however, the genotype and allele distributions of the control group varied significantly from the alcoholic group. When evaluating the separate roles of ADH2,

ADH3, and ALDH2 in predisposition to alcoholism within three tested models (codominance model, complete-dominance model, and partial-dominance model), they found that the ADH3 locus failed to show a significant effect (p = .23-56) on risk for alcoholism. Any previous reports of ADH3 having a significant protection against alcoholism was attributed to linkage disequilibrium of the ADH2 and ADH3 alleles (p < .013) based on the findings by Chen et al. (1999). The researchers, however, did find that the ADH2*1/*2 and ADH2*2/*2 genotypes showed highly significant protection against alcoholism (odds ratios [ORs], .12-.19) when compared to the ADH2*1/*1 genotype. When examining the combined effects of ADH2 and ALDH2, they found that ADH2*2/*2-ALDH2*1/*2 carriers have a 20 - 25-fold less risk for alcoholism than ADH2*1/*1-ALDH2*1/1 carriers. Most significantly, Chen et al. (1999) found that ALDH2*2/*2 homozygosity appeared to completely protect against the development of alcoholism while ALDH2*2/*1 heterozygosity displayed partial protection.

Chen and colleagues' (1999) study was one of the first studies to incorporate such a large number of subjects, assuring that their results could reflect valid population statistics among Han Chinese in Taiwan. This study was also one of the first studies that comprehensively tested the interactions between ADH2*2, ADH3*1, and ALDH2*2. Similar results have been duplicated in several related studies since then, showing that ALDH2*2/*2 homozygosity completely protects while ADH2*2/*2 homozygosity partially protects (~50%) against the development of alcoholism (Chambers, Marshall, Robinson, Maguire, Newton-Howes, & Chong, 2002; Garver, Tu, Cao, Aini, Zhou, & Israel, 2001; Kim et al., 2008; Thomasson et al., 1991).

CURRENT SYSTEMATIC REVIEW

While several studies have focused on the association between genetic influences and their role in determining alcohol consumption patterns among individuals, relatively few studies have considered the relationship between functional genetic polymorphisms and their ability to be used as a therapy for alcoholism via gene therapy. This systematic review paper focuses on studies that examine the functional polymorphisms of two liver enzymes (ADH and ALDH) and their ability to be used as a genetic foundation for the development of a potential, long-term treatment for alcoholism. In reviewing current research concerning the characteristics of ADH and ALDH, this paper considers how functional polymorphisms of the alcohol metabolism genes can be utilized as a protection against alcoholism and how the use of gene therapy can further the current treatment options for alcoholism.

METHOD

This systematic review was conducted using the procedures based on the Cochrane Group guidelines for systematic reviews (van Tulder, Furlan, Bombardier, Bouter, & the Editorial Board of the Cochrane Collaboration Back Review Group, 2003). A flow diagram of the search is displayed in Figure 1. Search terms were developed and integrated using Boolean logic to find studies targeting the specified population (e.g., adolescents, adults, humans, animals), intervention (e.g., gene therapy, adenovirus vector, RNA, antisense gene), and outcome measurement (e.g., alcoholism, alcohol behaviors, alcohol symptoms, alcohol use, ethanol intake). This systematic search was conducted through electronic database searches to identify meeting all inclusion criteria as of March 2018. As displayed in Figure 1, five studies met inclusion criteria.

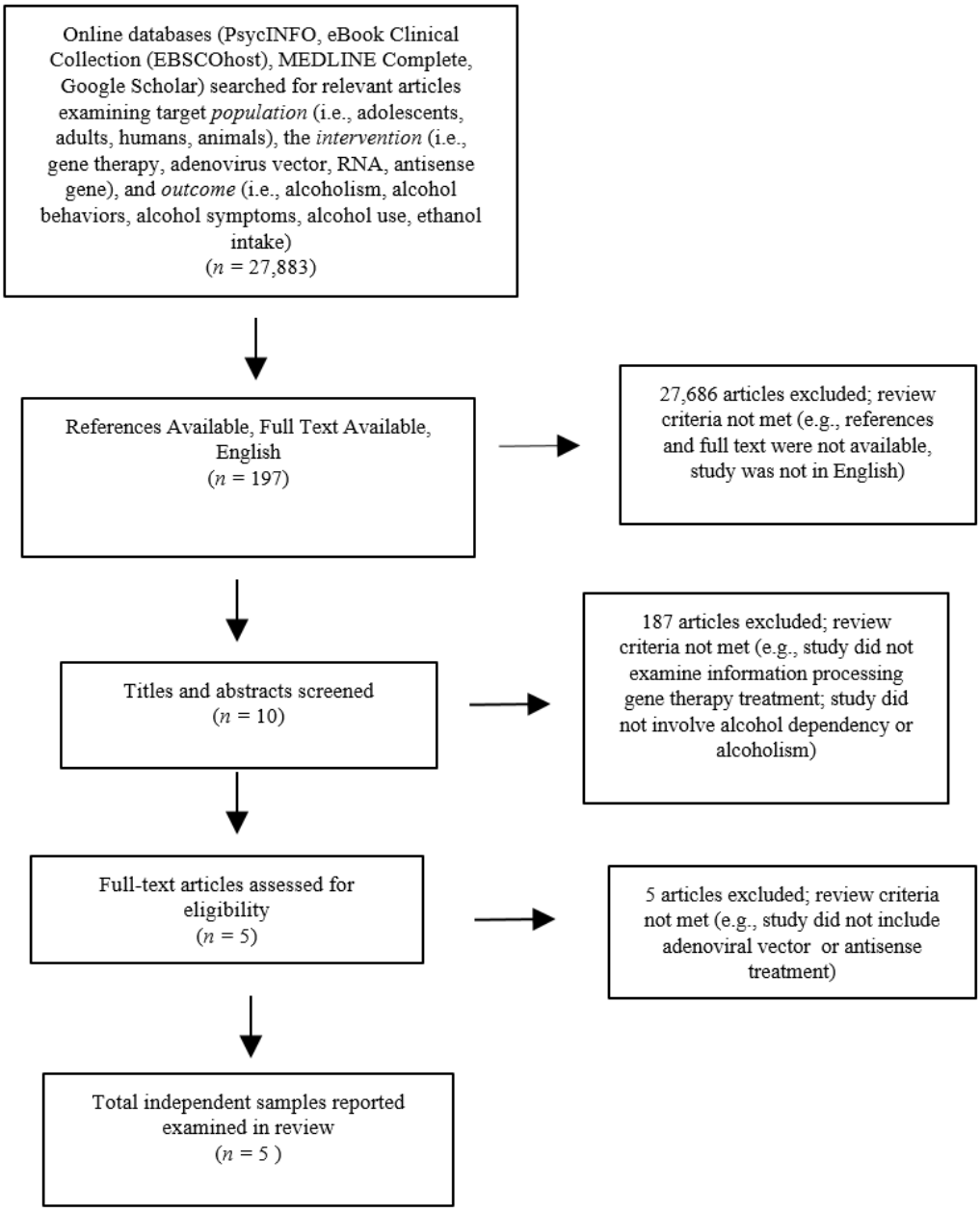

Figure 1. Flow diagram of article search

RESULTS

Table 1 summarizes study methods and results for the five studies that met all inclusion criteria. All studies included rats or rat cell lines; no studies included human participants. Four studies incorporated versions of adenoviral vectors to alter rat genomes while one study utilized antisense oligonucleotides to alter rat genomes. Of the

adenoviral vector studies, two examined the impact adenoviral vectors would have on ALDH activity on rats *in vivo* and *in vitro*, one examined the impact adenoviral vectors would have on ADH activity *in vivo*, and one examined the impact adenoviral vectors would have on both ADH and ALDH *in vivo*. The adenoviral vector studies included the use of *Aldh2* antisense vectors (AdV-AS) (n = 2), both *AdV-rADH-47His* and *AdV-rADH-47Arg* vectors (n = 1), and *AdV-ADH/asALDH2* vectors (n = 1) to test respective activity levels of ALDH2, ADH, and ADH/ALDH2 after the consumption of ethanol. The oligonucleotide study included in this systematic review examined the impact antisense phosphorothioate oligonucleotide (ASO) would have on ALDH activity *in vivo*.

All five studies found that respective enzyme activity was reduced after the introduction of either adenoviral vectors or oligonucleotides (Garver et al., 2001; Karahanian, Ocaranza, & Israel, 2005; Ocaranza et al., 2008; Rivera-Meza et al., 2010; Rivera-Meza, Quintanilla, & Tampier, 2012). Furthermore, all five studies showed increased acetaldehyde levels within infected cells after undergoing gene therapy. This reveals that gene therapy within the context of alcohol metabolism is able to mimic the reactions of polymorphic human cells within rat models.

Table 1. Study Methods and Results

Article	Conditions	Type	Ethanol Dose	*N*	Age or Weight	Ethanol Exposure Procedures	Measurement	Findings
Garver, Tu, Cao, Aini, Zhou, & Israel (2001)	Antisense Phosphorothioate oligonucleotide (ASO)	ASO-9 (intravenous administration)	1 g/kg ethanol	Male Lewis rats	Weight: 200-300 g	Rats treated for 4 days at 15 mg ASO-9/kg/day; oral ethanol administered on day 5 (1 g/kg); killed after 60 min	ALDH activity, mRNA expression, and acetaldehyde levels determined	• ASO-9 led to 38-45% reduction in ALDH2 activity and four-fold increase in acetaldehyde levels compared to control oligonucleotide • After 1 h of ethanol consumption, ASO-9 groups displayed 61% reduction in ethanol consumption ($p < 0.015$) compared to control • After 5 h of ethanol consumption, ASO-9 groups displayed 45% reduction in ethanol consumption ($p < 0.035$) compared to control
		ASO-9 (intraperitoneal administration)	6% vol/vol ethanol			Rats treated for 3 days at 24 mg/kg/day; ethanol administered on day 4		
	Control	Control oligonucleotide						
Karahanian, Ocaranza, & Israel (2005)	Adenoviral vector (MOI: 5)	*Aldh2* antisense (AdV-AS) vector	10mM ethanol	30 100 mm dishes of rat hepatoma cells	N/A	Rat hepatoma cells infected with vectors; cells incubated with ethanol for 1 h	ALDH2 activity and Acetaldehyde levels determined using a series of chemical reactions	• 48 h after transduction of *Aldh2* antisense gene led to reduction in ALDH2 activity (60-70%; $p < 0.001$) • *Aldh2* antisense gene led to eight-fold increase in acetaldehyde levels ($p < 0.001$)
	Adenoviral vector (MOI: 15)	*Aldh2* antisense (AdV-AS) vector						
	Control	*AdV*-noncoding (AdV) vector						
Ocaranza, Quintanilla, Tampier,	Adenoviral vector ($1 \, x \, 10^{12} \frac{vp}{kg}$)	Anti-*Aldh2* (AdV-AS) vector	10% ethanol	4 female rats/condition	2 months	60 d voluntary access to 10% ethanol; ethanol	Voluntary water and ethanol intake recorded; ALDH	• AdV-AS treated animals consumed 50% less ethanol during 34 day study compared

Karahanian, Sapag, & Israel (2008)	Control	AdV-C vector				withdrawn, AdV administered; Limited access paradigm: access to ethanol and water for 1 h each day for 34 days 72 h after adenoviral vector injection	activity and mRNA expression determined	to AdV-C treated animals ($p <$ 0.001) • Liver ALDH activity of AdV-AS treated animals was 85% reduced compared to AdV-C treated animals ($p < 0.002$)
Rivera-Meza, Quintanilla, Tampier, Mura, Sapag, & Israel (2010)	Adenoviral vector ($5 \times 10^{12} \frac{\text{vp}}{\text{kg}}$)	*AdV-rADH-47His* vector (mutant)	10% ethanol	5 female rats/condition	16 weeks Weight: 150-200 g	Limited access paradigm: Access to ethanol and water for 1 h each day for 13 days	Voluntary water and ethanol intake recorded; arterial acetaldehyde levels determined on day 21 using gas chromatography	• *AdV-rADH-47His* vector showed lower voluntary ethanol intake (50% reduction; $p < 0.001$) than animal with AdV-noncoding vector • *AdV-rADH-47Arg* vector showed lower voluntary ethanol intake (30% reduction; $p < 0.01$) than animals with AdV-noncoding vector • ADH activity in animals with *AdV-rADH-47His* vector (90%; $p < 0.01$) and *AdV-rADH-*47Arg vector (32%; $p < 0.01$) were higher than animals with AdV-noncoding vector
	Adenoviral vector ($5 \times 10^{12} \frac{\text{vp}}{\text{kg}}$)	*AdV-rADH-47Arg* vector (wildtype)						
	Control	*AdV*-noncoding vector						
Rivera-Meza, Quintanilla, & Tampier (2012)	Multiple expression cassette adenoviral vector ($3 \times 10^{12} \frac{\text{vp}}{\text{kg}}$)	*AdV-ADH/asALDH2*	10% ethanol	6 female rats/condition	16 weeks Weight: 150-200 g	Limited access paradigm: Access to ethanol and water for 1 h each day for 15 days before adenoviral vector injection; Access to ethanol and water for 1 h each day for 23 days 72 h after adenoviral vector injection	Voluntary water and ethanol intake recorded; arterial acetaldehyde levels determined using gas chromatography; ADH and ALDH2 levels determined	• *AdV-ADH/asALDH2* vector showed reduced voluntary ethanol intake for 3 weeks (60%; $p < 0.001$) compared to *AdV-C* vector animals • *AdV-ADH/asALDH2* vector showed increased levels of blood acetaldehyde with peak at 5-10 min after ethanol administration (400%; $p <$0.001) compared to *AdV-C* vector animals • *AdV-ADH/asALDH2* vector showed increased activity of ADH (176%; $p <$0.001) compared to *AdV-C* vector animals • *AdV-ADH/asALDH2* vector showed decreased activity of liver ALDH2 (24%; $p <$0.01) compared to *AdV-C* vector animals
	Control	*AdV-C*						

To determine ALDH2 activity in the Karahanian et al. (2005) study, total RNA was extracted from hepatoma cells. Antisense RNA was separated from endogenous RNA via reverse transcriptase (RT) reactions followed by PCR. ALDH2 activity was then measured. According to Karahanian et al. (2005), high levels of both antisense mRNA and sense mRNA was found in cells infected with the adenoviral vector carrying the antisense gene; however, antisense mRNA did not increase at higher multiplicities of infection (MOIs) of the viral vector. Furthermore, ALDH2 activity was reduced 60-70% ($p < 0.001$) forty-eight hours after transduction of the *Aldh2* antisense gene while acetaldehyde levels in cells infected with the antisense coding gene increased eight-fold ($p < 0.001$). Although ALDH2 activity showed slight differences at varying MOIs, acetaldehyde levels remained the same between the two MOI conditions. Karahanian and colleagues (2005) hypothesized that ALDH may begin removing acetaldehyde at high levels due to its higher K_m value, preventing further accumulation of acetaldehyde at a higher MOI. Overall, Karahanian et al. (2005) showed the administration of an adenoviral vector carrying an *Aldh2* antisense gene is able to increase the levels of acetaldehyde in rat cells metabolizing ethanol, successfully mimicking the effects of the functional polymorphism ALDH2*2 in humans metabolizing ethanol.

In a following study, Ocaranza and colleagues (2008) used rats bred as high alcohol drinkers as their subjects. Over the course of 2 months during which the rats were offered voluntary consumption of ethanol (10%) and water, all rats became alcohol dependent. Following this 2-month period, one group of rats ($n = 4$) had the therapeutic AdV administered via the tail vein while another group ($n = 4$) had a control AdV administered. All rats were then subjected to a 3-day withdrawal period during which they had no access to ethanol. After the withdrawal period, the two groups of rats were allowed access to a 10% ethanol solution (for

only one hour a day) and water (all day) for the following 34 days. Consumption patterns were recorded for all rats. Thirty-five days after the withdrawal period, the rats were euthanized and their liver ALDH2 activity was determined.

Ocaranza et al. (2008) observed that both groups of rats consumed intoxicating amounts of alcohol on the first day of ethanol re-access after the withdrawal period. However, over the next 34 days, the rats who had been administered the therapeutic AdV (AdV-AS) showed a dramatic reduction (50% lower than control AdV; $p = 0.001$) in ethanol intake while the rats who had been administered the control AdV (AdV-C) continued to consume ethanol at a high rate. The reduction in ethanol consumption was evident five days after the AdV-AS was injected and stabilized after 12 - 13 days. When analyzing liver ALDH2 activity, the researchers found that liver activity was reduced by 85% in rats that had received AdV-AS in comparison to the liver activity of those that had received AdV-C. Most importantly, this study found that gene therapy that mimics the low ALDH2 phenotype can elicit deterrence to high alcohol drinking even after high alcohol drinking has been established. The study done by Ocaranza and colleagues (2008) successfully showed that the single administration of the AdV carrying an anti-ALDH2 antisense gene was not only able to reduce liver ALDH2 activity, but it was also able to reduce alcohol intake for 34 days, which was the longest period of time studied up until that point. This study shows that gene therapy can be used as a possible long-term treatment option for alcohol-dependent animals and may be further developed into a long-term treatment option for humans with alcoholism.

To further investigate the mechanism by which alcohol metabolism polymorphisms protect against heavy alcohol intake, Rivera-Meza et al. (2010 & 2012) studied alcohol intake in rats transduced with the rat analog of the human ADH2*2 gene as well as with the rat analog of

the human ALDH2*2 gene in combination with the ADH2*2 gene. In the 2010 study, Rivera-Meza and colleagues cloned rat wild-type ADH (47Arg) and mutant ADH (47His) using methods similar to Karahanian et al. (2005) and Ocaranza et al. (2008) with the exception of using different promoter sequences and plasmids. Following this, adenoviral vectors AdV-rADH-47His and AdV-rADH-47Arg were formed, purified, and intravenously injected into each rat depending on which condition they were assigned (n = 6/condition). The rats were then allowed access to a 10% ethanol solution and water for 1 hour each day for 13 days before being euthanized to determine arterial acetaldehyde levels. According to Rivera-Meza et al. (2010), liver ADH activity within animals transfected with AdV-rADH-47His was six times higher than that within animals transfected with AdV-rADH-47Arg and 90% higher than that within animals transfected with the control AdV ($p < 0.01$). Additionally, animals that received the AdV-rADH-47His vector showed a significantly lower voluntary ethanol consumption level (50% reduction; p <0.001) than animals that received the control AdV. Overall, ADH2*2 showed that high levels of blood acetaldehyde occur only during the first few minutes of ethanol metabolism, resulting in a "burst" of acetaldehyde, which causes the reduction in voluntary ethanol intake. This "burst" is primarily caused by AdV-rADH-47His having a higher K_m for NAD+ at its active site and a 6-fold higher V_{max} than AdV-rADH-47Arg.

In order to expand upon their previous research, Rivera-Meza et al. (2012) developed a new therapeutic strategy to treat alcoholism by increasing ADH activity while also reducing ALDH2 activity to produce higher blood levels of acetaldehyde during ethanol metabolism. By doing so, the expression of both protective genes could be provided within a single adenoviral vector. In this study, Rivera-Meza and colleagues (2012) developed a multiple expression cassette adenoviral vector (AdV-ADH/asALDH2) that encoded both a fast rat ADH and an

antisense RNA against rat ALDH2. The adenoviral vector was formed using similar methods to that of which was previously described in the 2010 study. The rats in this study were allowed access to a 10% ethanol solution and water for 1 hour each day for 15 days before being intravenously injected with AdV-ADH/asALDH2. Seventy-two hours after the injection, rats were allowed access to ethanol and water for 1 hour each day for 23 days as researchers observed their ethanol intake. After the trial period, rats were subjected to seven days of abstinence from ethanol intake. After the abstinence period, the rats were given another dose of ethanol (1 g/kg) before being euthanized to measure acetaldehyde levels throughout the body.

Rivera-Meza et al. (2012) found that AdV-ADH/asALDH2 infected animals showed increased activity of ADH (176%; $p < 0.001$) and decreased activity of liver ALDH2 (24%; $p < 0.01$) compared to AdV-C vector animals. Likewise, AdV-ADH/asALDH2 vector showed reduced voluntary ethanol intake for 3 weeks (60%; $p < 0.001$) compared to AdV-C vector animals due to increased levels of blood acetaldehyde that peaked at 5-10 min after ethanol administration (400% increased; $p < 0.001$). Overall, this study showed that the paired increase of ADH activity and reduction of ALDH2 activity in the liver of alcohol-dependent rats is an effective method to reduce the voluntary ethanol intake in rodents, providing evidence of new therapeutic strategies for the treatment of alcoholism.

In a study by Garver et al. (2001), manufactured antisense phosphorothioate oligonucleotides (ASO), specifically ASO-9, was purchased and intravenously administered via a femoral venous catheter (15 mg ASO-9/kg/day) in rats for four days. On day five, an oral ethanol was administered (1 g/kg) and then the rats were euthanized one hour later in order to determine acetaldehyde levels and ALDH activity. Rats receiving the ASO-9 treatment showed a 50% reduction in ALDH2 mRNA compared to those receiving the control treatment. ALDH2

activity was reduced by 38-45% and displayed a four-fold increase in acetaldehyde levels after ethanol administration under ASO-9 treatment conditions in comparison to the rats in the control treatment.

To determine if ASO-9 could result in an aversion to ethanol and reduced ethanol consumption, Garver et al. (2001) surgically implanted rats with osmotic pumps which delivered ASO-9 at 24 mg/kg/day intraperitoneally. Three days after implantation with no access to water on the last night, rats were offered 6% ethanol as the only fluid and consumption was observed and measured at five hour intervals. During the first hour of ethanol presentation, initial ethanol consumption was similar in both the ASO-9 and control groups, resulting in 1.12 ± 0.9 g ethanol/kg (ASO-9) and 1.70 ± 0.68 g ethanol/kg (control). After the first hour, consumption was reduced significantly ($p < 0.015$) in the ASO-9 group (0.48 ± 0.23 g ethanol/kg) compared with the control group (1.22 ± 0.116 g ethanol/kg). Overall, a 45% reduction in ethanol consumption in ASO-9 treated animals was measured in comparison to animals receiving the control treatment ($p <0.035$).

All five studies included in this systematic review were able to utilize gene therapy techniques to mimic the functional polymorphisms of alcohol metabolism genes found primarily in East Asians within rat studies. As a result, all of the studies were able to lower their respective enzyme activities and elevate acetaldehyde levels after the administration of ethanol. In the *in vivo* studies, the elevated acetaldehyde levels resulted in an aversion to ethanol intake as shown by a reduction in alcohol consumption.

DISCUSSION

Several studies found that individuals who possess functional polymorphic alleles of the ADH and ALDH2 genes are genetically protected against alcohol abuse and alcoholism (Chen et

al., 1999; Higuchi et al., 1995; Ocaranza et al., 2008). In fact, individuals who are heterozygous ALDH2*2/ALDH2*1 carriers were protected against alcohol abuse and alcoholism by around 66 to 75% while homozygous ALDH2*2/ALDH2*2 carriers were nearly 100% protected (Chen et al., 1999; Ocaranza et al., 2008). ALDH2*2/*1 heterozygosity was found to display partial protection against alcoholism due to the presence of ALDH2*2 while individuals who were ALDH2*2/*1 heterozygous inactive and ADH2*1/*1 homozygous active still had a significantly high risk of alcoholism (Chen et al., 1999; Higuchi et al., 1995). However, individuals who were ALDH2*2/*1 heterozygous inactive and ADH2*2/*2 homozygous inactive were largely protected against risk of alcoholism.

In the study done by Ocaranza and colleagues (2008), liver ALDH2 activity was reduced via a single administration of the AdV carrying an anti-ALDH2 antisense gene; the therapeutic AdV was also able to reduce alcohol intake for 34 days in rats, which was the longest period studied up until that point. This study along with the studies done Karahanian et al. (2005) and Garver et al. (2001) reveal that gene therapy that mimics the polymorphic nature of ALDH can be used as a possible long-term treatment option for alcohol-dependent animals. Furthermore, these studies showed that deterrence can be elicited after high alcohol drinking has been established, elevating the ability of gene therapy to be used as a possible treatment option for humans with alcoholism. According to Karahanian et al. (2005), therapeutic reduction of ALDH2 activity in the liver should be the primary aim in order to allow other tissues within the lungs, upper respiratory tract, and upper gastrointestinal tract to retain their ability to metabolize acetaldehyde. In doing so, risk of alcoholism in addition to risk of developing oropharyngolaryngeal, esophageal, and lung cancer due to the carcinogenic effects of acetaldehyde can be lowered.

The two studies done by Rivera-Meza et al. (2010 & 2012) incorporated gene therapy focused on ADH involvement in alcohol metabolism. In doing so, they found that ADH polymorphisms play a large contributing factor in ethanol aversion due to a burst in acetaldehyde production. Additionally, Rivera-Meza et al. (2012) found that simultaneously increasing ADH activity and reducing ALDH2 activity was able to reduce the voluntary ethanol intake in rodents, which provides evidence of new therapeutic strategies for the treatment of alcoholism.

In order to bridge the gap between gene therapy used in animal studies and any potential future studies involving human subjects, more studies that consider the relationship between functional genetic polymorphisms and their interactions with developmental stages and variations in environmental context need to be pursued so that our understanding of how polymorphisms such as ADH and ALDH work to protect humans against alcoholism throughout their lifespan can be improved. Likewise, a greater number of animal studies that include larger sample populations need to be tested before implementing similar gene therapy techniques in humans to decrease the risk of alcoholism to ensure that methods are safe and that results are able to be reproduced and to the same magnitude.

Future animal studies should test the effect of gene therapies similar to the ones described within the studies included in this systematic review with the addition of longer periods of abstinence during the withdrawal phase to more closely mimic the behavior of recovering alcoholics. To test this, helper-dependent AdVs (HD-AdV) that are able to express therapeutic genes for up to two years without the use of endogenous viral genes can potentially be used in future rat and nonhuman primate studies (Rivera-Meza et al., 2012). The large cloning capacity and ability to accommodate multiple transgenes and lower chronic toxicity of helper-dependent adenoviruses allow them to be a plausible candidate vector for future liver-directed gene therapy.

Furthermore, due to the intrinsic hepatotoxic effects of ethanol and the prolonged exposure to ethanol within the gene therapy studies discussed in this systematic review, future studies should be aware of and examine increases of acute and chronic hepatotoxicity of adenoviral vector administration in animals.

Future studies involving human subjects should focus on the gene-environment interaction of ADH/ALDH and sociocultural factors/developmental stages. Likewise, important sociocultural variables such as mental health, personality traits, and relationships with parents need to be considered. These variables require that future studies utilize a longitudinal design so that fluctuations in the aforementioned variables are able to be observed and assessed effectively. Future studies should also strive to include other ethnicities (Middle East Asians, Caucasians, African Americans, etc.) when examining the gene-environment interaction seeing that familial, cultural, and peer interactions play a significant role in determining the protective effects of genes on alcoholism. These problems may be addressed within longitudinal studies that focus on alcohol behaviors and thought processes within a variety of ethnically different communities. As suggested by Irons, Iacono, Oetting, and Mcgue (2012), future studies should also examine measures such as levels of access to alcohol with the house, family/peer alcohol use, and overall beliefs on alcohol. Examining the gene-environment interaction is critical to any future understanding of how to better our currently existing treatments for alcoholism in both young adolescents and adults, and it is a necessary component to consider when bridging the gap between animal studies and future gene therapy techniques developed for humans.

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