Can Gene Therapy Be Used to Prevent Cancer? Gene Therapy for Aldehyde Dehydrogenase 2 Deficiency

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Abstract

Approximately 8% of the world population and 35 to 45% of East Asians are carriers of the hereditary disorder, aldehyde dehydrogenase 2 (ALDH2) deficiency. ALDH2 plays a central role in the liver to metabolize ethanol. With the common E487K variant, there is a deficiency of ALDH2 function; when ethanol is consumed, there is a systemic accumulation of acetaldehyde, an intermediate product in ethanol metabolism. In ALDH2 deficient individuals, ethanol consumption acutely causes the "Alcohol Flushing Syndrome" with facial flushing, tachycardia, nausea and headaches. With chronic alcohol consumption, ALDH2 deficiency is associated with a variety of disorders, including a remarkably high risk for aerodigestive tract cancers. Acetaldehyde is a known carcinogen. The epidemiologic data relating to the association of ALDH2 deficiency and cancer risk are striking: ALDH2 homozygotes that are moderate to heavy consumers of ethanol have a 7- to 12-fold increased risk for esophageal cancer, making ALDH2 deficiency the most common hereditary disorder associated with an increased cancer risk. In this review, we summarize the genetics and biochemistry of ALDH2, the epidemiology of cancer risk associated with ALDH2 deficiency, the metabolic consequences of ethanol consumption associated with ALDH2 deficiency and gene therapy strategies to correct ALDH2 deficiency and its associated cancer risk. With the goal of reducing the risk of aerodigestive tract cancers, in the context that ALDH2 is a hereditary disorder and ALDH2 functions primarily in the liver, ALDH2 deficiency is an ideal target for the application of adeno-associated virus-mediated liver directed gene therapy to prevent cancer.

Introduction

Aldehyde dehydrogenase 2 (ALDH2) deficiency, an autosomal recessive disorder involving ethanol metabolism, is one of the most common hereditary disorders, affecting 560 million people, representing 8% of the world population[1, 2]. ALDH2 deficiency is very common in the East Asian population, with an allele frequency of 35–45%[3, 4]. Ethanol metabolism involves two major NAD-dependent enzymes, alcohol dehydrogenase (ADH) and ALDH2[5]. With ethanol consumption, ADH converts ethanol to acetaldehyde and then ALDH2 metabolizes acetaldehyde to acetate[6] (Figure 1). Mutations in the *ALDH2* gene (GenBank Nt_029419), most commonly E487K (referred to as the *ALDH2*2* allele, the normal allele is *ALDH2*1*), result in a deficiency of ALDH2 function with subsequent systemic accumulation of acetaldehyde following ethanol consumption.

The consequences of ethanol consumption in individuals with ALDH2 deficiency are significant. Acutely, ethanol consumption by ALDH2 deficient individuals causes the "Alcohol Flushing Syndrome," with facial flushing, tachycardia, nausea and headaches[1]. With chronic ethanol ingestion, ALDH2 deficiency is associated with a variety of neurologic, endocrine, cardiovascular and dermatologic disorders, aberrant drug metabolism, and the subject of this review, a marked increased risk of upper aerodigestive tract cancer of the oral cavity pharynx, larynx and esophagus (reviewed in Chen et al[2]).

The increased risk of ALDH2 deficiency for upper digestive tract cancers, particularly esophageal cancer, is one of the highest risk factors for cancer. Of those who chronically drink alcohol, both ALDH2 homozygotes and heterozygotes are at risk of developing esophageal cancer[1, 7] and the risk increases with the extent of drinking[8, 9]. Strikingly, *ALDH2*2/*2* homozygotes that drink alcohol and also smoke cigarettes have a 50-fold risk of esophageal cancer[10, 11]. The focus of this review is the opportunity to use gene therapy to reduce the high risk for aerodigestive cancer associated with ALDH2 deficiency.

Aldehyde Dehydrogenase 2

The human ALDH group of enzymes includes 19 isozymes responsible for metabolizing alcohol byproducts. Of this group, there are two major ALDH isoforms, the cytosolic ALDH1 and mitochondrial ALDH2[6]. Mitochondrial ALDH2 is the most efficient in metabolizing alcohol-derived acetaldehyde because of its extremely low Km (~0.2 μM) for acetaldehyde, 900-fold lower compared to cytosolic ALDH1[12].

The human *ALDH2* gene located on chromosome 12q24 codes for a 517-amino acid polypeptide[13]. The ALDH2 protein is a 56 kDa tetramer with four identical subunits that each contain a catalytic domain, a coenzyme or NAD^+ binding domain, and an oligomerization domain[14]. Together, the tetrameric structure contributes to normal functioning of enzymatic ALDH2. The most common *ALDH2*2* mutation representing 33 to 51% of all *ALDH2* mutations associated with ALDH2 deficiency, results in dysfunction of the ALDH2 tetramer[15]. With the *ALDH2*2/*2* genotype, the E487K mutation disturbs hydrogen bond stabilization and modifies the αG helix and the arginine 475 loop. Disruptions in these interactions impair the structure of the NAD^+ binding site and rearrange residues in the catalytic domain[16–18]. Because of these modifications, the Km for NAD^+

increases by 200-fold such that ALDH2 is unable to combine with NAD^+ or coenzymes and is limited in enzymatic activity[16].

The amino acid configuration in the tetrameric structure (E487 or K487) affects functionality of the ALDH2 enzyme. Enzymatic activity is 100% when all 4 subunits have the E487 amino acid but decreases if the subunit sequence is modified. If the tetramer has a single subunit with the K487 variant (1/4) and three normal E487 remaining subunits, the tetramer has 48% reduced ALDH2 activity. The presence of the K487 variant in two subunits (2/4) and three subunits (3/4) reduces ALDH2 activity to 12 and 5%, respectively. When all four subunits (4/4) have the K487 variant, ALDH2 activity is 4%[19]. With ethanol consumption, the consequence of reduced ALDH2 activity is the systemic accumulation of acetaldehyde, resulting in the clinical manifestations of the deficiency state.

Prevalence

The *ALDH2*2* allele has a high prevalence in East Asians[20]. Among these population groups, the *ALDH2*2* allele is observed in 25 to 45% of Chinese, Korean and Taiwanese origin and ~50% of Japanese, while nearly absent in other population groups such as Caucasians and African Americans[20, 21] (Table I). For example, the *ALDH2*2* allele frequency is 0.0004 in Latino/Admixed American, 0.0003 in South Asian, 0.0002 in African/African American, 0.0001 in European (Finnish), 0.00002 in European (non-Finnish) and is absent in the Ashkenazi Jewish population[3].

Because other population groups also experience alcohol flushing syndrome in response to alcohol consumption, studies have looked at other variants in the *ALDH2* gene that cause the ALDH2 deficiency state. Healthy donors from a blood bank in Mexico City, identified two novel ALDH2 variants (P92T and V304M). The Exome Aggregation Consortium (ExAC) database (formerly ExAC, now gnomAD database) identified five missense variants (P92T, V304M, I41V, T244M and R338W) present at a frequency greater than 0.5%. These variants are at low allele frequencies (~2.5–2.6%) among Latinos compared to the high frequency of the E487K classic *ALDH2*2* variant among East Asians[15]. In all genome-wide association studies for alcohol use disorders, the *ALDH2*2* polymorphism is observed at a noticeably greater significance compared to any other variant[22].

Metabolic Consequences of the Major ALDH2 Deficiency Variant with Ethanol Consumption

*ALDH2*2* carriers have a dysfunctional ALDH2 enzyme, which limits the capacity of the liver to eliminate acetaldehyde and results in the accumulation of acetaldehyde in the blood following alcohol consumption[23, 24]. Because of the reduced ALDH2 activity, *ALDH2*2/*2* and *ALDH2*1/*2* individuals have blood acetaldehyde levels that are markedly higher than *ALDH2*1/*1* individuals following alcohol consumption[25]. In normal individuals, blood acetaldehyde levels range from 2 to 20 μM[24]. In *ALDH2*2/*2* homozygotes who consume low to moderate levels of ethanol, blood acetaldehyde levels peak at 23 to 81 μM and in *ALDH2*1/*2* heterozygotes, 8 to 26 μM[26].

Acetaldehyde is highly reactive and can directly interact with DNA, introducing point mutations[27, 28]. Since acetaldehyde can directly bind to DNA, it can interfere with DNA

replication, leading to double-stranded breaks[24]. Some of these chromosomal aberrations are repaired by proteins of the homologous recombination repair pathway including Rad51 and phosphorylated H2A histone family member X (γH2AX), which are both used clinically as biomarkers for the detection of DNA damage[24].

Acetaldehyde forms acetaldehyde-DNA adducts that are mutagenic and potentially carcinogenic[29]. Several of the common mutagenic DNA adducts include N^2-ethylidene-2'-deoxyguanosine (N^2-ethylidene-dG; often quantified by its reduced, stable form as N^2-ethyl-2'-deoxyguanosine [N^2-Et-dG][30]), N^2-(2,6-dimethyl-1,3-dioxan-4-yl)-deoxyguanosine and α-methyl-γ-OH-propano-deoxyguanosine (Cr-PdG)[31]. Of these, N^2-ethylidene-dG, the most abundant DNA adduct derived from exposure to acetaldehyde, is a common biomarker used to detect acetaldehyde-derived DNA damage[32–34]. N^2-Et-dG is detectable in leukocytes of human heavy ethanol drinkers with the *ALDH2*2* genotype[35–37 38]. Cr-PdG has the capacity to generate secondary lesions that arise as DNA-protein crosslinks or DNA inter-strand crosslinks[27]. Because these lesions impair DNA replication and promote cell death, Cr-PdG is considered genotoxic and highly mutagenic[27, 39]. Together, N^2-Et-dG and Cr-PdG initiate replication errors and mutations in oncogenes or onco-suppressor genes and promote carcinogenesis[27, 39].

In addition to DNA adducts, acetaldehyde protein adducts can be generated by the interaction of acetaldehyde with either lysine residues or the alpha amino group of N-terminal amino acids[27]. These adducts then alter the structure and function of the protein and, in the cases of enzymes, disrupt enzymatic activity[27]. For example, acetaldehyde forms adducts with the DNA repair mechanism enzyme, O6-methylguanine methyltransferase[27]. Because the formation of this protein adduct disables the DNA repair function of the enzyme, carcinogenesis may be induced[27].

Several lines of evidence link ALDH2 deficiency, alcohol consumption, systemic elevation in acetaldehyde levels, DNA damage and the increased risk for esophageal cancer. Yukawa et al[40] identified significantly higher levels of DNA adducts (N^2-Et-dG, Cr-PdG, and N^2-ethylidene-dG) in ALDH2-deficient Japanese alcoholics. In animal studies, Matsuda et al[32] observed elevated levels of N^2-ethylidene-dG in the liver of heterozygous (*Aldh2$^{+/-}$*) and homozygous ALDH2 knockout (*Aldh2$^{-/-}$*) mice consuming 20% ethanol for 5 weeks[32, 34]. Yukawa et al[37] observed significantly elevated esophageal N^2-ethylidene-dG levels in *Aldh2$^{-/-}$* mice compared to control mice after intraperitoneal administration of ethanol, consistent with the concept that circulating ethanol-derived acetaldehyde levels contribute to induction of esophageal DNA damage. Amanuma et al[34] studied whether DNA damage was induced in the esophagus of ALDH2 wildtype (*Aldh2$^{+/+}$*) and *Aldh2$^{-/-}$* mice following chronic consumption of 10% ethanol for 8 weeks. Elevated levels of γ-H2AX and N^2-ethylidene-dG were observed in the esophagus of *Aldh2$^{-/-}$* mice following ethanol consumption indicating a significant degree of DNA damage compared with *Aldh2$^{+/+}$* mice[34]. These findings of elevated N^2-ethylidene-dG are similar to those observed in ALDH2-deficient Japanese alcoholics[40].

Consistent with the data indicating that systemic acetaldehyde generated by chronic ethanol ingestion will induce cancer-related changes in the esophagus, elevated serum acetaldehyde

levels and esophageal damage and adducts were observed in $Aldh2^{-/-}$ knockout and E487 knockin ($Aldh2^{E487K+/+}$) mice chronically administered alcohol in the drinking water[41–43]. $Aldh2^{-/-}$ and $Aldh2^{E487K+/+}$ mice had significantly higher serum acetaldehyde levels compared to wild type mice after 12 wk ethanol consumption. ALDH2 also metabolizes other aldehydes including malondialdehyde (MDA), a reactive aldehyde derived from oxidative stress with implications in DNA adduct formation and mutagenesis. MDA levels in the liver were significantly higher in $Aldh2^{-/-}$ and $Aldh2^{E487K+/+}$ mice drinking ethanol for 12 weeks compared to wild type mice[43].

Risk for Esophageal Cancer Associated in Humans with ALDH2 Deficiency and Ethanol Consumption

The combination of ALDH2 deficiency and ethanol consumption, the focus of this review, are major risk factors for the development of aerodigestive cancers[1, 5, 7–11, 25, 28, 29, 34, 37, 44–76]. In addition to aerodigestive cancers, there is extensive literature detailing the association of ALDH2 deficiency and ethanol consumption with an increased risk for many other disorders, including other cancers and non-cancer related diseases such as cardiovascular disease, diabetes, and neurodegenerative disease. For details, there are several reviews[2, 10, 49, 53, 57–59, 65, 73, 75, 77, 78].

Upper aero digestive tract cancers, including esophageal, oral and laryngeal cancer, together constitute 3.5 to 4.0% of all malignancies[71]. Individuals homozygous for *ALDH2*2* that drink alcohol have a 7- to 12-fold increased risk of upper aerodigestive tract cancers[1, 7, 71]. Consistent with the knowledge that the ALDH2 tetramer with two K487 subunits has only 12% normal activity[19], the increased risk for esophageal cancer includes not only *ALDH2*2* homozygotes, but also *ALDH2*2* heterozygotes. High hazard ratios are found in *ALDH2*2* heterozygote Japanese men who consumed more than 23 g of ethanol on occasion and drink more than 5 times per week[74]. Heterozygote heavy drinkers, or those that consumed more than 46 g of ethanol on occasion and drank 5 days or more per week also have a high odds ratio[74]. Association studies between risk for esophageal cancer and amount of alcohol consumption demonstrate a strong association by a significant odds ratio with the rs671 polymorphism in *ALDH2*2* homozygotes or heterozygotes in both moderate drinkers and heavy drinkers[44, 55, 56, 62, 68, 69, 76].

The risk for esophageal cancer in association with ALDH2 deficiency is dependent on the extent of alcohol consumption. *ALDH2*2* homozygous individuals that avoid alcohol consumption are protected from the high risk associated with esophageal cancer[61]. The risk of ALDH2 deficient individuals developing esophageal cancer increases 3-fold from those who never consumed alcohol to moderate drinkers and 2-fold from moderate drinkers to heavy drinkers[54, 70, 72]. Meta-analysis of the association between ALDH2-deficient individuals and esophageal cancer in Japan and China[60] reported an odds ratio of 1.28 in never drinkers, 3.12 in moderate drinkers, and 7.12 in heavy drinkers[60]. The *ALDH2*1/*2* genotype is associated with a high risk of esophageal cancer in Taiwanese, Chinese, and Japanese moderate drinkers (odds ratio 4.74–6.21) and heavy drinkers (9.21–9.75). This risk is lower in regions of Mainland China moderate drinkers (1.98) or heavy drinkers (1.31)[60]. The highest risk of esophageal cancer is observed in *ALDH2*2* homozygote cigarette

smokers that consume alcohol who have an odds ratio 50, with a 25-year earlier onset of esophageal carcinoma[10, 11].

Consequences of Excess ALDH2 Activity Drug Resistance

While ALDH2 deficiency and alcohol consumption is associated with increased aerodigestive cancer risk, excess ALDH2 activity is linked to drug resistance[79–81]. Various studies report a correlation between ALDH2 overexpression and multi-drug resistance against common cancer drugs such as antimetabolic agents and microtubule inhibitors[79–81]. In microtubule-inhibitor resistant head and neck cancer cells, upregulated ALDH2 expression is reduced by treatment with disulfiram (Antabuse), an ALDH2 inhibitor, and copper, a combination treatment used to override drug resistance, causing apoptosis of the cancer cells[82]. When *ALDH2* is silenced with small interfering RNA (siRNA), cytotoxicity of anticancer drugs, such as Taxol, is enhanced and drug resistance is inhibited in lung and head and neck cancer cell lines[82].

Prior Therapeutic Strategies Relevant to ALDH2

Attempts at therapies to enhance ALDH2 enzymatic activity have used enzymatic activators or substances to mitigate acetaldehyde elevation. Alda-1, an ALDH2 activator, enhances ALDH2 enzymatic activity and inhibits alcohol-induced oxidative stress[83, 84]. In human *ALDH2*2* knockin mice, Alda-1 significantly increased liver ALDH2 levels and reduced esophageal DNA damage levels after ethanol consumption[85]. In rats, *ALDH2*2* recombinant homotetramers treated with Alda-1 had an 11-fold increase in ALDH2 enzymatic activity[71, 83]. Although no ALDH2 activators have moved forward to clinical trials, together, these studies indicate that Alda-1-mediated activation of ALDH2 theoretically could serve as a therapeutic intervention in reducing the toxic effects of acetaldehyde[83].

"Essential AD2" is a nutritional supplement reported to increase ALDH2 activity and reduce acetaldehyde levels, thereby reducing the flushing reaction experienced by ALDH2 deficient individuals following alcohol consumption. Subjects with ALDH2 deficiency receiving essential AD2 daily for 28 days had decreased blood acetaldehyde levels following alcohol use[86].

ADH inhibitors impede the metabolism of alcohol to acetaldehyde. For example, 4-methylpyrazol (4-MP) competitively inhibits the oxidation of ethanol to acetaldehyde by ADH and therefore reduces the ethanol elimination rate. When ALDH2 deficient individuals and individuals with normal ALDH2 ingested 4-MP orally, after 2 hours, both the ALDH2 deficient and normals groups showed a reduction (38% and 46%, respectively) in ethanol elimination rate, with a rise in blood ethanol levels and a decrease in acetaldehyde levels. The flushing response was suppressed in both groups following treatment[87].

In a mimic of the naturally occurring ALDH2 deficiency state, a variety of strategies have focused on reducing alcohol intake by inhibiting ALDH2 activity, including the FDA approved drug disulfiram (Antabuse)[88], daidzein[89], kudzu extract[90], puerarin[91], bitter herbs (gentian, tangerine peel)[92] and decinol[93]. These remedies increase acetaldehyde and induce

symptoms similar to the ALDH2 deficiency state after alcohol intake with the goal of reducing alcohol use. The effect of these compounds on cancer risk have not been evaluated.

Gene Therapy to Prevent Cancer Risk Associated with ALDH2 Deficiency

Since ALDH2 deficiency is a hereditary disorder that primarily manifests in the liver, it should be amenable to adeno-associated virus (AAV)-mediated gene therapy to augment the inactive mitochondrial ALDH2 enzyme with a normal *ALDH2*1* allele. By doing so, ALDH2 enzymatic activity could be restored and acetaldehyde generated following ethanol consumption metabolized to non-toxic acetate, theoretically reducing the risk for esophageal cancer.

Matsumura et al[43, 94] used two murine models to assess the feasibility of using gene therapy to prevent esophageal cancer associated with ethanol consumption in the ALDH2 deficiency state. The *Aldh2* knockout mouse (*Aldh2$^{-/-}$*) has undetectable ALDH2 protein or enzymatic activity[41]. The *Aldh2* E487K knockin mouse (*Aldh2$^{E487K+/+}$*, also called *ALDH2*2* knockin) has the *ALDH2*2* (E487K) lysine mutation found in ALDH2-deficient individuals inserted in the mouse *Aldh2* gene, resulting in significantly reduced levels of ALDH2 enzymatic activity[42].

To demonstrate that AAV-mediated gene therapy can correct the ALDH2 deficiency state and prevent the accumulation of acetaldehyde after ethanol consumption and the resultant acetaldehyde-induced esophageal DNA abnormalities, an AAVrh.10 serotype gene transfer vector coding for normal human ALDH2 was generated (Figure 2A). Two studies were carried out, one to assess the acute effects of ethanol[94] and the other the chronic effects of ethanol[43]. Both studies used the *Aldh2$^{-/-}$* and *Aldh2$^{E487K+/+}$* models. The following describes the results with the *Aldh2$^{E487K+/+}$* model; for details, see Matsumura et al[43, 94]. By delivering an AAVrh.10hALDH2 to murine hepatocytes, wild-type liver enzymatic activity was normalized, and serum acetaldehyde levels were significantly lower after acute alcohol consumption (Figure 2B, C). When *Aldh2$^{E487K+/+}$* mice were treated with an AAVrh.10hALDH2, there was a significant reduction in γH2AX positive cells in the esophageal epithelium and significant reduction in the number of N^2-Et-dG adducts formed in the esophageal DNA when compared with AAVrh.10control-treated *Aldh2$^{E487K+/+}$* mice (Figure 3). DNA adducts and DNA damage are precursors to the formation of esophageal cancer, thus prevention of their formation by treatment with AAVrh.10hALDH2 suggests that restoration of ALDH2 expression and function in the liver could help mitigate the risk of esophageal cancer in ALDH2 deficient individuals.

Alternative Gene Therapy Strategies

Other gene therapies have focused on ALDH2 with therapies designed to reduce chronic ethanol intake and binge drinking. Instead of correction of the ALDH2 deficiency state, these therapies were developed to inhibit the ALDH2 enzyme function to induce the common Alcohol Flushing Syndrome associated with ALDH2 deficiency, with the goal of deterring alcohol intake.

Sanchez et al[95] delivered an *ALDH2* short hairpin RNA (shRNA) packaged in AAV serotype 2 under the control of the U6 promoter *in vitro* to HEK-293T cells and HepG2

cells. The goal was to inhibit mitochondrial ALDH2 enzymatic activity *in vitro* using a self-complimentary AAV encoding a shRNA specific to the human *ALDH2* transcript[95]. To observe the effect of the ALDH2 vector on acetaldehyde levels, human cell lines were exposed to ethanol, resulting in increasing acetaldehyde levels. If applied to humans, this inhibitory shRNA approach would induce the ALDH2 deficiency state. While this may discourage alcohol consumption because it would establish the Alcohol Flushing Syndrome, it theoretically would also result in an increased risk for the ALDH2 deficiency associated disorders, including esophageal cancer.

In another gene therapy, a lentivirus vector augmenting ALDH2 expression resulted in reduction of ethanol intake. Karahanian et al[96] administered a lentiviral vector coding for rat *ALDH2* directly into the ventral tegmental area of 3- to 4- month-old female rats. Following the single injection, ethanol intake was greatly reduced for a 45-day period[96]. A similar result in rats has been observed with the ALDH2 activator Alda-1[97].

Translation of AAV-based ALDH2 Gene Therapy to the Clinic

Based on the preclinical data that a single intravenous administration of AAVrh.10-based delivery of the human *ALDH2* coding sequence to ALDH2 deficient mice prevents chronic alcohol ingestion from inducing systemic acetaldehyde accumulation and consequent accumulation of DNA adducts and damage[43], it is relevant to consider how these preclinical observations could be translated to humans with ALDH2 deficiency. In this context, we propose a two-step clinical design.

First, we suggest that the phase I clinical study to demonstrate safety and biochemical efficacy of AAV-based gene therapy enroll ALDH2-deficient homozygotes with a history of daily ethanol consumption who smoke cigarettes and have esophageal biopsy evidence of pre-cancerous lesions (moderate to severe dysplasia)[98, 99]. This population has an extraordinarily high risk for development of esophageal cancer. In addition to the marked increased risk of esophageal cancer in *ALDH2*2* homozygotes who continue to drink[1, 11, 65, 100–106], the presence of moderate to severe esophageal dysplasia is associated with a 15.8-fold increased risk of developing esophageal cancer over 3.5 yr[107]. Prior to gene therapy, each subject will be assessed for the biochemical response to oral administration of a standard amount of ethanol, with subsequent assessment of serum levels of acetaldehyde and acetate, quantification of breath ethanol levels and facial flushing. The following day, the subject will be administered the gene therapy, with doses determined by the results of a toxicology study in experimental animals. Four wk after administration, the subject will be rechallenged with the same standard ethanol dose, with assessment for the same parameters as prior to therapy. We expect that pre-therapy, the ethanol challenge will result in elevated ethanol and acetaldehyde levels and low acetate levels. However, post-therapy, the ethanol challenge should lead to elevated ethanol and acetate levels, but low or no increase in acetaldehyde levels. Pre-therapy facial flushing will be increased with ethanol challenge, but post-therapy facial flushing should be minimal.

Second, successful demonstration of safety and biochemical efficacy in the Phase I trial will provide support for initiating a Phase II clinical trial focused on prevention of esophageal cancer. The specific design of the Phase II trial will depend on the results of the Phase I

trial, but because the risk for esophageal cancer is so high, the optimal risk-benefit group would be cigarette smoking *ALDH2*2* homozygotes that continue to drink alcohol and had esophageal biopsy evidence of moderate to severe dysplasia. Compared to a placebo group, the primary outcome would be reduction in the development of esophageal cancer over the study period.

Conclusion

In this review, we have outlined the risk of developing esophageal cancer in those with ALDH2 deficiency that consume alcohol. Extensive epidemiologic studies have linked alcohol consumption in ALDH2-deficient individuals with an increased risk of esophageal cancer. The risk of cancer is largely due to the accumulation of acetaldehyde in ALDH2 deficiency because these individuals are unable to metabolize acetaldehyde to non-toxic acetate. As systemic acetaldehyde accumulates following alcohol consumption, the aldehyde acts as a genetic modifier by binding DNA and disrupting cellular repair mechanisms. Acetaldehyde-DNA and acetaldehyde-protein adducts form that are potentially carcinogenic. In the context that ALDH2 deficiency is a hereditary disorder and that the liver is the dominant site of ethanol metabolism, AAV gene therapy to augment normal ALDH2 enzymatic function in the liver provides an opportunity to use gene therapy to prevent the risk of esophageal cancer in ALDH2 deficient individuals who continue to consume alcohol.

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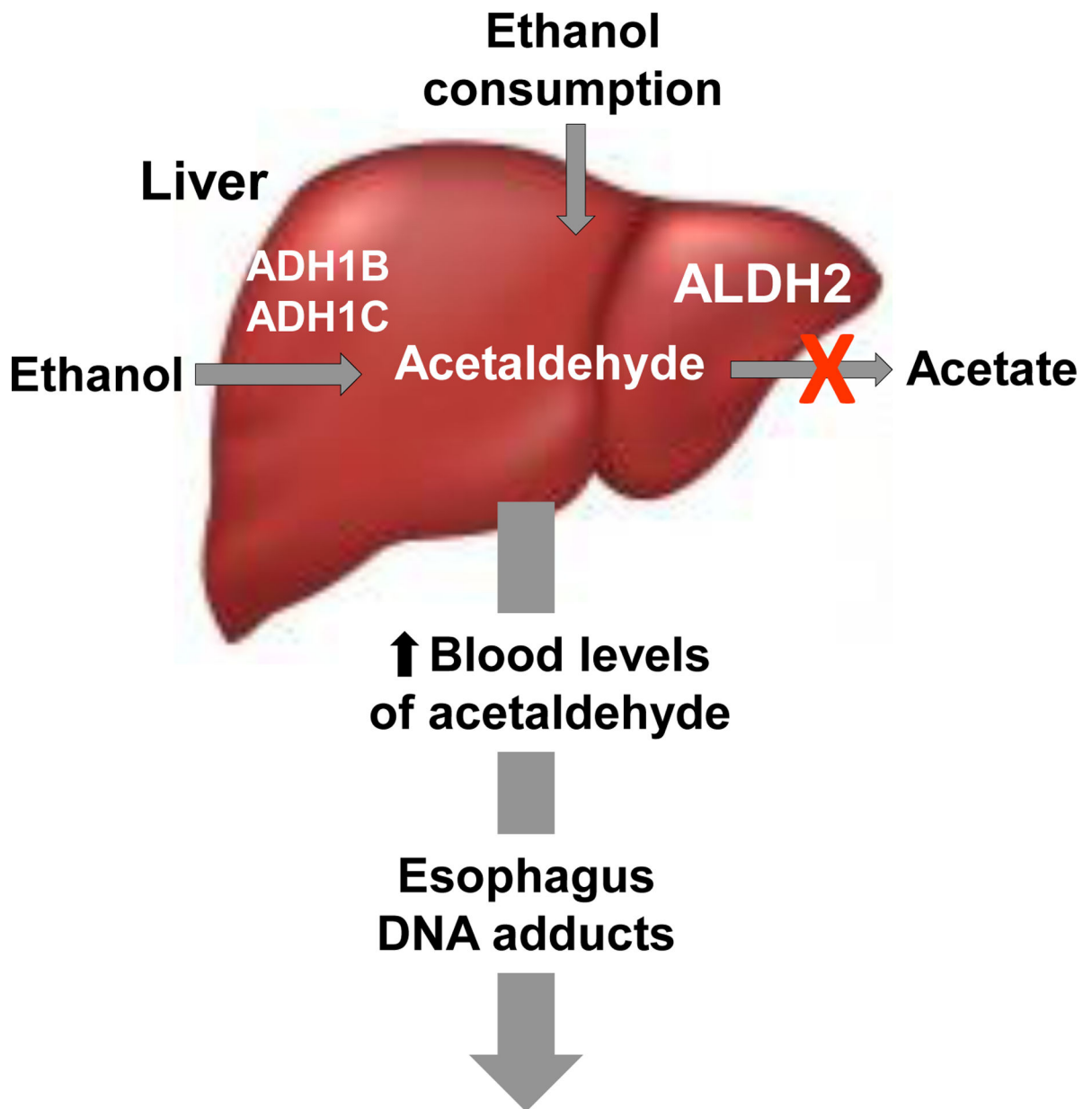
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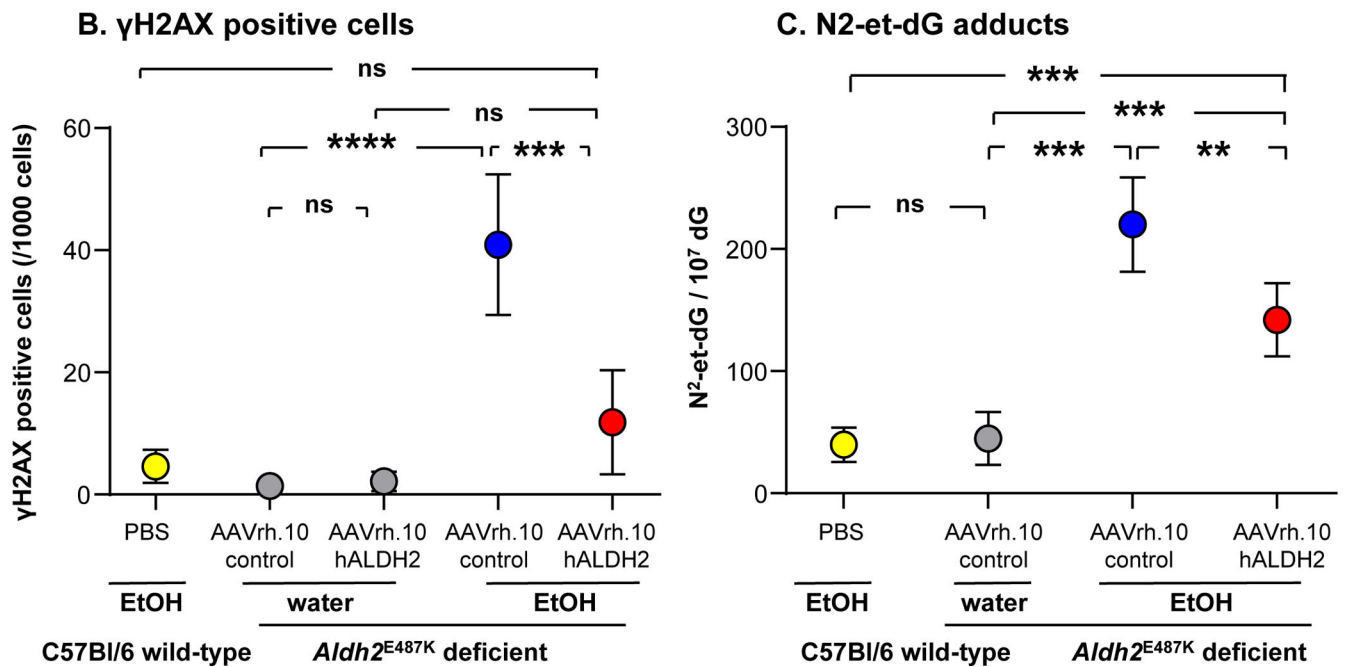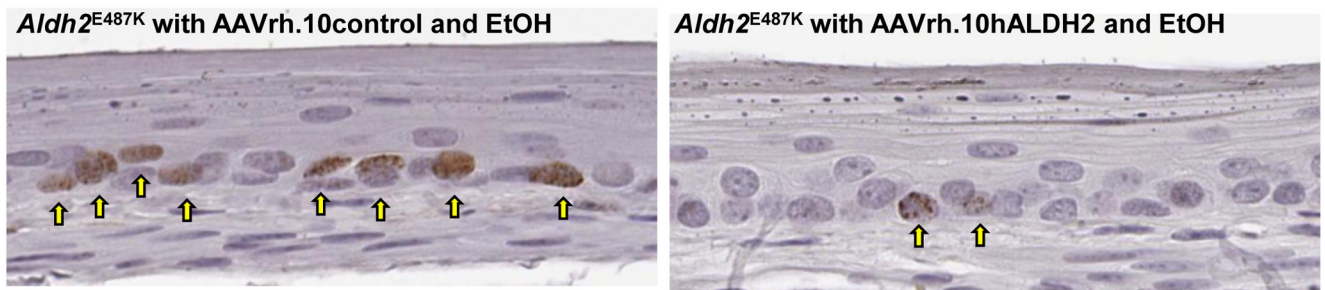
Figure 1.
The high risk for esophageal cancer in ALDH2 deficiency results from a combination of the ALDH2 deficiency state and ethanol consumption. The liver is the major site of ethanol metabolism. Alcohol dehydrogenases (ADH1B, 1C) convert ethanol to acetaldehyde, which is metabolized by ALDH2 to acetate. With mutations in ALDH2, there is a deficiency in liver ALDH2 enzymatic activity and with ethanol intake acetaldehyde accumulates. Acutely, this causes the "Alcohol Flushing Syndrome," but with chronic alcohol consumption, chronic elevated levels of acetaldehyde are associated with DNA damage and the accumulation of DNA adducts in the esophagus, predisposing to esophageal cancer.
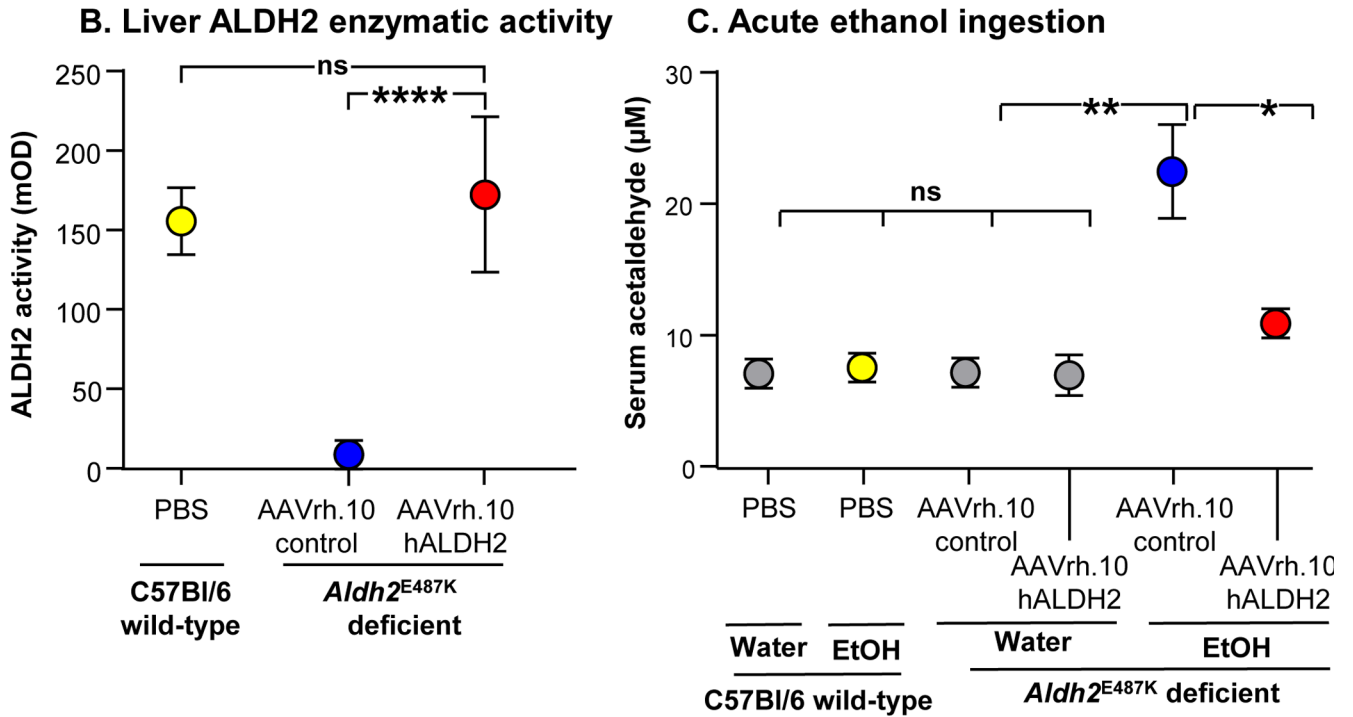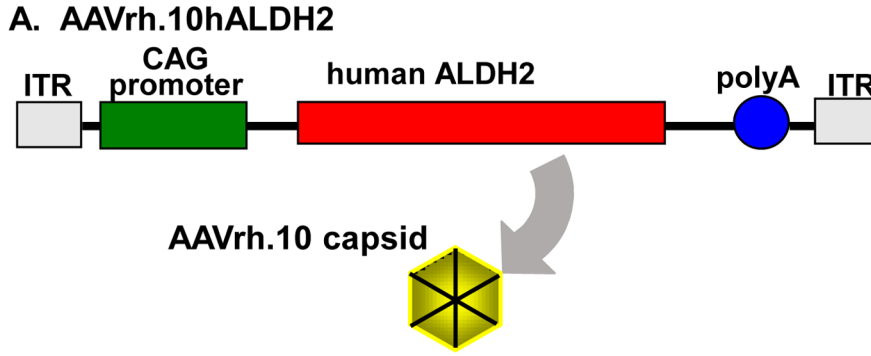
Figure 2.

AAVrh.10hALDH2 correction of ALDH2 deficiency in the $Aldh2^{E487K+/+}$ murine model.
A. AAVrh.10hALDH2 construct. The expression cassette including the AAV2 inverted terminal repeats (ITR), CAG promoter, and the human ALDH2 coding sequence (hALDH2) packaged in the AAVrh.10 capsid. **B.** AAVrh.10hALDH2-mediated liver human ALDH2-driven enzymatic activity in $Aldh2^{E487K+/+}$ mice. Four wk following intravenous administration, $Aldh2^{E487K+/+}$ deficient mice had ALDH2 enzyme activity similar to that of wild-type mice (10^{11} genome copies). **C.** AAVrh.10hALDH2-mediated prevention of serum acetaldehyde accumulation in $Aldh2^{E487K+/+}$ mice administered ethanol acutely. Four wk post-administration of AAVrh.10hALDH2, mice were given water or ethanol (4 g/kg body weight) by intragastric gavage and serum was collected 6 hr later for acetaldehyde quantitation. Values are presented as means ± SEM. *p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. Data from Matsumura et al[94] with permission.

A. Immunohistochemistry of γH2AX positive cells in esophageal epithelium

B. γH2AX positive cells

C. N2-et-dG adducts

Figure 3.
Effect of AAVrh.10hALDH2 therapy on DNA damage and adducts in the esophageal epithelium after 12 wk chronic ethanol exposure. $Aldh2^{E487K+/+}$ mice were intravenously administered AAVrh.10hALDH2 or AAVrh.10control (10^{11} genome copies), and C57Bl/6 mice were administered PBS. Four wk after vector administration, mice were challenged with water or ethanol in water for 12 wk. **A.** Representative immunohistochemical staining of γH2AX positive cells in esophageal epithelium of $Aldh2^{E487K+/+}$ mice. **B.** Quantitation of γH2AX positive cells in esophageal epithelium $Aldh2^{E487K+/+}$ after chronic ethanol exposure. **C.** DNA was extracted from whole esophagus and N^2-et-dG adducts were quantified by LC-MS/MS. Values are presented as means ± SEM. ** p<0.01, *** p<0.001, **** p<0.0001. Data from Matsumura et al[43], with permission.

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Table I.

Prevalence of *ALDH2*2* Allele in Global Populations

Population	Population allele frequencies (AF)[1]		
	gnomAd	1000 Genomes	NCBI ALFA
East Asian	0.2554	0.174	0.244
Latino/Admixed American	0.0004094	0.003	0
South Asian	0.0002698	0	0.001
African/African-American	0.0002073	0.002/0	0.009/0
Finnish	0.00008095	0	*
European	0.00002404	0	0.0000822
Ashkenazi Jewish	0.000	0	*

[1] Population allele frequencies for the rs671 single nucleotide polymorphism (*ALDH2*2* E487K mutation) reported in National Center for Biotechnology (NCBI) databases including gnomAD, 1000 Genomes Project Phase 3, and NCBI Allele Frequency Aggregatory (ALFA).

* Allele frequency not reported

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