



# Role of ALDH2 in Hepatic Disorders: Gene Polymorphism and Disease Pathogenesis

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## Abstract

Aldehyde dehydrogenase 2 (ALDH2) is a key enzyme of alcohol metabolism and it is involved in the cellular mechanism of alcohol liver disease. *ALDH2* gene mutations exist in about 8% of the world's population, with the incidence reaching 45% in East Asia. The mutations will result in impairment of enzyme activity and accumulation of acetaldehyde, facilitating the progression of other liver diseases, including non-alcoholic fatty liver diseases, viral hepatitis and hepatocellular carcinoma, through adduct formation and inflammatory responses. In this review, we seek to summarize recent research progress on the correlation between *ALDH2* gene polymorphism and multiple liver diseases, with an attempt to provide clues for better understanding of the disease mechanism and for strategy making.

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## Introduction of gene polymorphisms in aldehyde dehydrogenase 2

### Function of aldehyde dehydrogenase 2 in human beings

The aldehyde dehydrogenases play a key role in the metabolism of toxic aldehydes. Some are produced in human

bodies, such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA), while others were obtained from the environment, like formaldehyde, acrolein, and ethyl.<sup>1,2</sup> As a member of the ALDH superfamily, ALDH2 is the most sensitive isoform to irreversible inactivation and is also the most sensitive to inactivation by toxics, such as 4-HNE.<sup>1</sup> This enzyme could metabolize acetaldehyde (ACH) to acetate irreversibly in a redox reaction (Fig. 1).<sup>3</sup> Disturbances in the expression of *ALDH2* will dampen its metabolic capacity and result in accumulation of ACH consequently. Based on its electrophilic feature, ACH could bind with biomolecules such as proteins or DNA and destroy cell integrity, which contributes to the development of various human diseases,<sup>4</sup> such as endocrine disorders, cardiovascular diseases, pulmonary diseases, oral cancers, gastrointestinal cancers, Fanconi anemia, and dermatitis.<sup>5–7</sup>

### ALDH2 gene and polymorphisms

ALDH2 is a polypeptide consisting of 517 amino acids, principally expressed in the liver but also in other organs, such as heart, kidney, muscle, and brain.<sup>8</sup> Its coding gene is located on chromosome 12 (12q24.2), which is 44 kilobases in length and comprises 13 exons.<sup>9</sup> After translation, the expressed protein is transported to the mitochondrial matrix to participate in dehydrogenase, esterase and reductase reactions in liver and fat tissues particularly. Studies of the human genome have shown 19 kinds of functional *ALDH* genes in total, with a wide range of expression and substrate specificities, among which the *ALDH2* gene has the highest expression and exclusively harbors existence of genetic polymorphisms.<sup>1</sup>

As it encodes a key enzyme for alcohol metabolism, *ALDH2* also has an important functional single nucleotide polymorphism (SNP), the *rs671-Glu504Lys* variant, which has significantly reduced activity compared to the wild type.<sup>10</sup> The *rs671* variant exists in 30–45% of East Asians (Chinese, Japanese, and Korean) and 8% of the world's population.<sup>11,12</sup> The incidence of this mutation in China is as high as 37–59%.<sup>13–16</sup> Nowadays, the *rs671*SNP locus at exon 12 is of special concern in worldwide research. According to sequencing detection,<sup>17</sup> a G→A point mutation is prone to occur at exon 12, causing the original glutamic acid (Glu) to be replaced by lysine (Lys), whose mutation is named *ALDH2Glu504Lys* (SNPrs671).

*ALDH2rs671* SNPs are composed of three genotypes: GA, AA and GG. GA is a heterozygous mutation, also named as *ALDH2\*1/\*2* (Glu/Lys). AA is a homozygous mutation, also known as *ALDH2\*2/\*2* (Lys/Lys). GG is the normal allele, without mutation (Fig. 2). The majority of studies on these genotypes have confirmed that the GA genotype has 10–20% of the enzyme activity compared wild type, while the

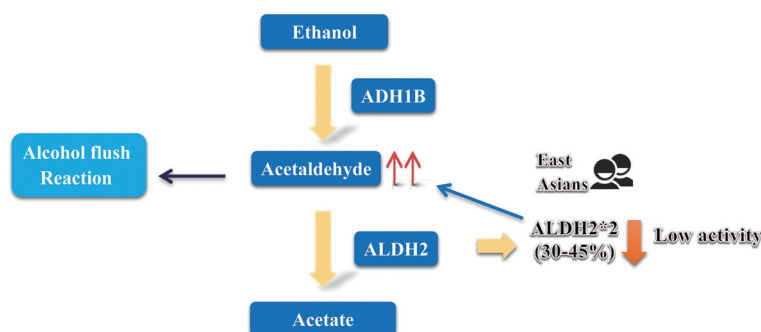
**Keywords:** Aldehyde dehydrogenase 2; Gene polymorphisms; Alcoholic liver disease; Non-alcoholic fatty liver disease; Viral hepatitis; Hepatocellular carcinoma.

**Abbreviations:** 4-HNE, 4-hydroxy-2-nonenal; ACH, acetaldehyde; ALD, alcoholic liver disease; ALDH2, aldehyde dehydrogenase 2; ALT, alanine aminotransferase; AMPK, AMP-activated protein kinase; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CYP2E1, cytochrome P4502E1; GGT, γ-glutamyl transpeptidase; Glu, glutamic acid; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HSC, hepatic stellate cell; Lys, lysine; HDL-C, high-density lipoprotein cholesterol; HIF-1α, hypoxia-inducible factor-1 alpha; ISGs, interferon-stimulated genes; MDA, malondialdehyde; NAFLD, non-alcoholic fatty liver disease; NF-κB, nuclear factor-kappa B; PP2A, protein phosphatase 2A; ROS, reactive oxygen species; SNP, single nucleotide polymorphism; SREB-1, sterol regulatory element binding protein-1 pathway; STAT, signal transducer and activator of transcription; TG, triglyceride.

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**Fig. 1. Alcohol metabolism and enzymes that strongly impact alcohol consumption.** The *ALDH2*\*2 variant exists in 30–45% of East Asians (Chinese, Japanese, and Korean), which has low activity.

AA genotype loses more than 96% of the enzymatic activity. As a result, individuals with GA or AA mutations could show up to 6 or 19 times greater ACH concentrations, respectively, as compared with wild type after alcohol intake.<sup>18</sup>

### Distribution of *ALDH2* alleles in different populations

The genotype frequencies of the *ALDH2* gene polymorphisms vary among different races. The rare *ALDH2*\*2 allele has been observed in Caucasians, Africans and Southeast Asians but it is widely present in East Asians.<sup>19,20</sup> There is a report of this mutation being found in about 560 million people of East Asian descent and reducing enzymatic activity by approximately 60% to 80% in *ALDH2*\*1/\*2 heterozygotes.<sup>19</sup> Among East Asians, the *ALDH2* allele frequencies are diverse among Japanese, Korean, and Chinese. In China, the *ALDH2*\*2 gene frequency in some Chinese aboriginal populations (e.g., Korean, Uighur, Zhuang and Olunchun) is lower compared to the Chinese Han population. In the Chinese Han population, the *ALDH2*\*2 allele frequency is 17% to 29%, the proportion of individuals with *ALDH2*\*1/\*2 heterozygotes is 36% to 44%, and the proportion of individuals with *ALDH2*\*2/\*2 homozygotes is 7% to 8%.<sup>21,22</sup>

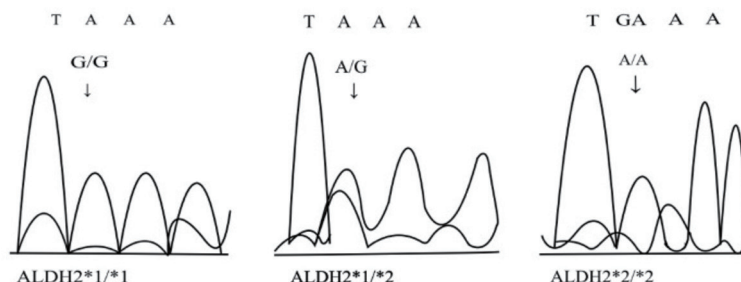
### Related liver diseases

#### Alcoholic liver disease

Alcoholic liver disease (ALD) is a direct outcome of chronic ethanol consumption and is considered as an important health problem worldwide. ALD encompasses a broad spectrum of liver injuries, including steatosis, fibrosis, cirrhosis, and alcoholic hepatitis.<sup>23</sup> The incidence of ALD has been increasing yearly because of the rapid boom in alcohol con-

sumption in many developing countries over the past decade.<sup>24</sup> The prevalence of ALD in related individuals,<sup>29</sup> though its polymorphism does not contribute to alcohol dependence in the Turkish population.<sup>30</sup> Regardless of homozygous AA or heterozygous GA status, both guarantee elevated ACH level after alcohol drinking. A single-center study from the Fifth Medical Centre of the General Hospital of the Chinese People's Liberation Army reported that only 2.3% of ALD patients have the *ALDH2*\*2 allele, compared with 14.5% of the proportion of healthy controls (281 and 535 controls; odds ratio [OR] of 0.13 and 95% confidence interval [CI] of 0.07–0.24).<sup>31</sup> In Korea, Lee et al.<sup>32</sup> found that the *ALDH2*\*1 allele is associated with a higher frequency of alcoholic cirrhosis ( $p=0.001$ ). Likewise, a meta-analysis of 12 studies found that people with the *ALDH2*\*1 allele are more likely to go on to develop alcoholic liver cirrhosis compared with those with either the *ALDH2*\*1/\*2 or *ALDH2*\*2/\*2 genotype.<sup>33</sup> Based on the activity of the enzyme after gene mutation, *ALDH2*\*2/\*2 should have produced a poor protective effect of ethanol; however, it brings some body information, such as facial flushing, reminding those with *ALDH2*\*2/\*2 to be alert to alcohol intake and usually leading to little excessive ethanol consumption.<sup>34</sup> On the contrary, without the gene reminder, those with *ALDH2*\*1 are not aware of consuming excessive alcohol.

The protection from the *ALDH2* Glu504Iys polymorphism has also been verified by Liu's team,<sup>35</sup> whose result demonstrated that individuals carrying this polymorphism are protected from alcohol drinking, with a 4-fold decrease in risk. Ma et al.<sup>36</sup> and Li et al.<sup>16</sup> also provided further evidence that



**Fig. 2. Genotypes of *ALDH2*rs671.**

**Table 1. Recent clinical studies on the relationship between the ALDH2 polymorphism and ALD**

Year	Conclusion	Reference
2001	The <i>ALDH2</i> *2 gene protects against the development of alcoholism.	32
2012	Strong protective effect of the <i>ALDH2</i> 504lys (*2) allele against alcoholism and alcohol-induced medical diseases in Asians.	16
2015	The absence of the <i>ALDH2</i> *2 allele in both alcoholics and controls suggests that this polymorphism does not contribute to alcohol dependence in the Turkish population.	30
2015	ALDH2 plays a beneficial role in ameliorating chronic alcohol intake-induced hepatic steatosis and inflammation, through regulation of autophagy.	37
2016	People with the <i>ALDH2</i> *1 allele are more likely to go on to develop alcoholic liver cirrhosis compared with those with the <i>ALDH2</i> *1/*2 or <i>ALDH2</i> *2/*2 genotype.	33
2016	Polymorphisms in <i>ALDH2</i> exerted significant indirect effects on hepatocellular carcinoma risk, mediated through alcohol drinking.	35
2016	The <i>ALDH2</i> Glu504Lys polymorphism and 'alcohol flush' are not harmless in the study's Asian population.	36
2017	Individuals who carry at least one copy drink typically less and are protected against heavy alcohol use and alcohol use disorders.	34
2018	Patients with the <i>ALDH2</i> 504lys variant were less associated with ALD compared to those with <i>ALDH2</i> 504glu.	31
2019	ALDH2 deficiency is associated with elevated acetaldehyde and glucocorticoids post-alcohol consumption, thereby inhibiting T cell activation and hepatitis.	37

Abbreviations: ALD, alcohol liver disease; ALDH2, aldehyde dehydrogenase 2; Glu, glutamic acid; Lys, lysine.

the mutation and "alcohol flush" are not harmless in this Asian population. In other words, the *ALDH2* gene mutation is a protective factor in the alcohol-drinking population in East Asia, while it is weaker in European and African populations.<sup>34</sup> In fact, the Eastern culture encourages or challenges people to drink more alcohol in social activities, and sometimes people with flushing may not be able to escape or reject such alcoholism.

Aerobic glycolysis is involved in alcohol metabolism, which could be inhibited by a known factor: corticosterone. As is shown in the animal experiment of Gao's team,<sup>37</sup> a higher level of serum corticosterone is detected in ethanol-fed *Aldh2*(-/-) mice, compared to the wild type mice. Gao's team<sup>37</sup> also found that acute alcohol drinking in humans was related to elevated plasma glucocorticoid levels in human subjects, with higher levels in those with inactive ALDH2 than active ALDH2. To conclude, the progress of aerobic glycolysis is impaired by ethanol, especially in those with *ALDH2*\*2. Meanwhile, they succeeded in restored concanavalin A-mediated hepatitis via blockade of corticosterone. Therefore, aerobic glycolysis-related signaling pathways may be a key factor. Interestingly, the authors found that glucose metabolism in T cells could be disrupted by ACH through inhibition of the aerobic glycolysis-related signal pathways. In addition, weakened autophagy is involved and compromised lysosomal activity will lead to abnormal stacking of ethanol or acetaldehyde by-product including protein or DNA adducts. Guo *et al.*<sup>38</sup> reported that observations both *in vivo* and *in vitro* are in favor of a beneficial role of ALDH2 in alcohol intake-facilitated fatty liver and inflammation through autophagy regulation (Table 1<sup>16,30-37</sup>).

The traditional hypothesized pathway is through oxidative stress. ALDH2 dramatically attenuates hepatic oxidative stress induced by chronic alcohol intake and favors a role of oxidative stress in ethanol- and ALDH2-elicited hepatic responses, by restoring autophagy and reopening autophagy flux. Additional ethanol consumption will increase the production of NADH/NAD<sup>+</sup>, and reactive oxygen species (ROS) in the mitochondrial electron transport chain. Then, ROS is able to activate nuclear factor-kappa B (NF-κB) and

its downstream proinflammatory signal, and correspondingly aggravate inflammation and hepatocyte damage.<sup>39-41</sup> Moreover, Zhong *et al.*<sup>42</sup> selected mitochondrial ALDH2 as a promising therapeutic target for ALD. They said that it accelerates aldehyde clearance and reverses hepatic steatosis and apoptosis in mice. Therefore, artificial modulation of *ALDH2* expression may be a potential therapeutic intervention for alcoholism and ALD in the future.<sup>43,44</sup>

As mentioned above, variants in ALDH2 decrease the rate of ACH conversion to acetate because it blocks its ability to remove ACH and results in a strong aversive reaction. Therefore, if we can find a medium to intervene this mechanism and develop a blocker, we will alleviate this effect. It is also suggested that physicians should pay attention to explore the potential immunosuppressive therapy in alcoholics.

### Non-alcoholic fatty liver disease

It has become more and more accepted that non-alcoholic fatty liver disease (NAFLD) stands for not just a single type of liver disease but the hepatic manifestation of complicated metabolic dysfunctions. NAFLD covers a wide range of liver pathologies, including steatosis, steatohepatitis, fibrosis/cirrhosis and liver failure.<sup>14,45</sup> Nowadays, NAFLD has become the leading cause of chronic liver diseases on earth and its global prevalence is appropriately 25%.<sup>46-49</sup> Prevalence rates of NAFLD were estimated to be 22.4%, 24.13%, 23.71%, 25%, 31% and 32% in China, the USA, Europe, Japan, the Middle East, and South America, respectively.<sup>14,24,50,51</sup> In the USA, NAFLD is estimated to be the most common cause of chronic liver disease, affecting between 80 and 100 million individuals, among whom nearly 25% progress to non-alcoholic steatohepatitis.<sup>14</sup> A recent report of data from the National Health and Nutrition Examination Survey ranging from 1988 to 2010 indicated that modest alcohol consumption (7–21 g/day) is associated with decreased mortality among patients with NAFLD.<sup>52</sup> In contrast to the studies of 58,927 patients with NAFLD in Korea, even moderate drink-

ers (10–29.9 g/day) exhibited an increased tendency to progress to fibrosis compared with non-drinkers.<sup>53</sup> Whether consuming moderate alcohol could be a lifestyle intervention in the treatment requires further investigation.

In China and East Asia, genome-wide association studies found that *ALDH2 rs671* is a susceptible gene locus for obesity and significantly associated with increased body mass index and visceral fat deposition.<sup>10,11</sup> A control study reported that a significant accumulation of the 4-HNE protein adduct and a significant up-regulation of *ALDH2* protein expression are found in the two groups of non-alcoholic steatohepatitis patients, suggesting that *ALDH2* plays a role in combating non-alcoholic steatohepatitis oxidative stress. We have reason to speculate that *ALDH2* gene mutation will reduce the oxidative stress protection response of liver recovery in non-alcoholic steatohepatitis patients. On the contrary, Alda-1, as an activator of *ALDH2*, suppresses PINK1/PARKIN-mediated mitophagy and its usage in apoE-mice has led to improvement in the degree of arteriosclerosis and hepatic steatosis, indicating that activation of *ALDH2* can improve NAFLD on the condition of mitochondrial injury caused by vinyl chloride.<sup>54</sup> Alda-1 protects against liver damage under these conditions via increasing clearance of aldehydes and preserving mitochondrial respiratory function.

In the field of biomedicine, researchers have found that inhibition of *ALDH2* enhances the ACH-mediated hepatocyte sterol regulatory element binding protein-1 (SREB-1) pathway activity and promotes triglyceride (TG) deposition in the liver. A cohort study<sup>55</sup> conducted by Japanese researchers followed 341 patients who never or seldom drank or drank less for 4–6 years and found that the incidence of NAFLD is higher in carriers of the mutant gene *ALDH2\*2* than in non-carriers. Carriers of *ALDH2\*2* with elevated  $\gamma$ -glutamyl transpeptidase (GGT) levels ( $>25.5$  IU/L) have a significantly increased risk of NAFLD. A recent Japanese study investigated the association of *ALDH2rs671* genotype with liver disease in 1,768 alcohol-dependent Japanese men. They found that the ineffective *ALDH2* Glu/Lys genotype increases the ratio or regression coefficient of age- and alcohol-adjusted fatty liver, ketonuria and serum high-density lipoprotein cholesterol (HDL-C) level, and reduces liver cirrhosis and serum TG level. Through logistic regression analysis, Chinese scientists also found that the GGT level in carriers of the GA/AA type is significantly higher than that of the GG type, suggesting that carriers of the GA/AA type are more prone to suffer liver cell damage and more severe fatty changes than carriers of the GG type. Nevertheless, another animal study suggested that *ALDH2*-deficient individuals may be resistant to steatosis and blood alanine aminotransferase (ALT) elevation but be more prone to liver inflammation and fibrosis following alcohol consumption.<sup>15</sup> Furthermore, a human experiment showed that the prevalence of elevated ALT level increases with the accumulation of components of metabolic syndrome and that the correlation between active *ALDH2* and elevated ALT level is sensitive.<sup>56</sup> Their logistic regression analysis also revealed that body mass index, TG level, and *ALDH2* genotype are associated with ALT elevation. This result coincides with the findings of the genome-wide association studies.

To conclude, the relationship between *ALDH2* and the NAFLD disease spectrum has begun to enter the laboratory stage but it needs a large number of research studies to clarify the connection.

### Viral hepatitis

Although the global incidence of viral hepatitis, hepatitis B virus (HBV) infection mainly, is going down, it continues

to play an important role in developing countries.<sup>57</sup> There are approximately 257 million people with chronic HBV infection globally, including 68% in Africa and the Western Pacific, according to a World Health Organization report.<sup>58</sup> In China, chronic hepatitis B (CHB) and chronic hepatitis C (CHC) affect 90 million and 10 million people, respectively. In developed countries such as the USA, Japan, and the European Union, the prevalence of HBV is much lower (0.71–1.17%), but the prevalence of hepatitis C virus (HCV) (1.10–1.56%) is higher than in China (HBV: 6.52%; HCV: 0.72%). In 2016, the Global Health Sector Strategy on viral hepatitis called for elimination of viral hepatitis as a major public health threat by 2030.<sup>59</sup> However, unlike other liver diseases, the relationship between viral hepatitis and *ALDH2* remains unclear.

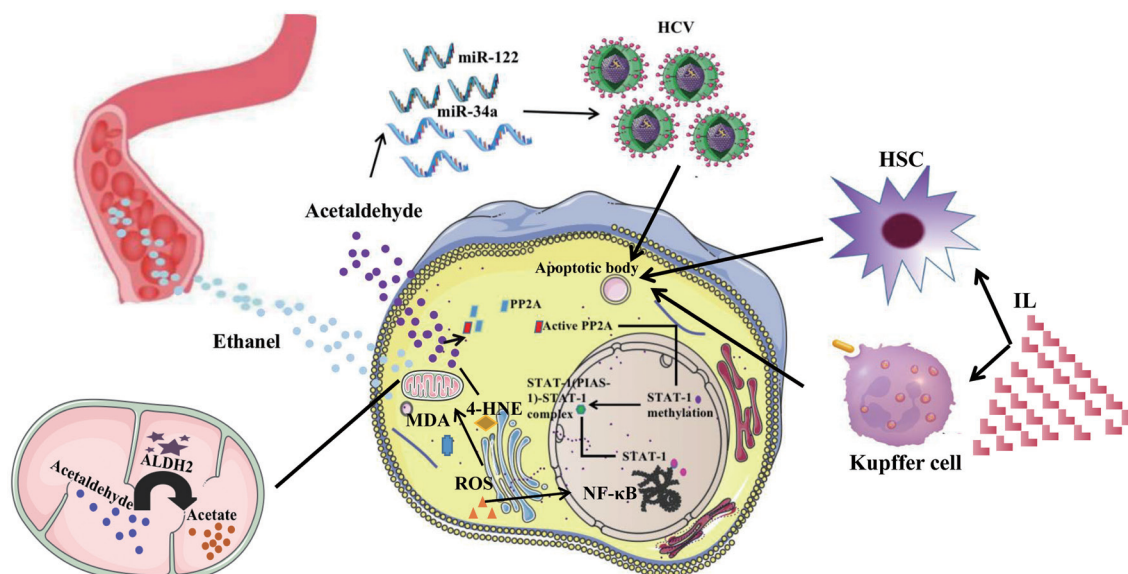
HCV infection is an important cause of chronic liver disease, with nearly 71 million chronically infected people worldwide.<sup>57</sup> HCV and alcohol intake are both risk factors for accelerated fibrosis progression,<sup>60</sup> and alcohol use in the setting of HCV infection is correlated with increased rates of fibrosis progression.<sup>61</sup> Based on previous studies,<sup>62,63</sup> the correlation between *ALDH2* and HCV could be explained by the two following aspects: enhanced virus replication and immunity suppression.

For one thing, the metabolite ACH could help to activate the expression of miR-122 and miR-34a, both able to stimulate HCV replication.<sup>64</sup> Correspondingly, a large number of virus products brought about by strong virus replication will promote hepatocellular apoptosis. Apoptosis has a secondary amplification effect on the viral lethality in the liver, which not only delays virus clearance but also aggravates liver cell damage. And, then, Kupffer cells and hepatic stellate cells (HSC) are driven by interleukins to aggregate and participate in the phagocytosis and clearance of apoptotic bodies.<sup>62</sup> This process will accelerate the inflammatory responses and fibrogenesis in the liver. Meanwhile, ACH could increase the activity of protein phosphatase 2A (PP2A).<sup>65</sup> PP2A could reduce methylation of signal transducer and activator of transcription (STAT)-1 and formation of the protein inhibitor of the activated STAT-1 PIAS-1-STAT-1 complex.<sup>64</sup> Ultimately, the damage will enhance destruction of STAT-1 caused by HCV, thereby increasing the apoptosis (Fig. 3).

For another, some scientists have claimed that impairment of immunity is a probable cause. Ethanol exposure enhances the inhibitory effect of HCV on innate immunity, thereby activating the spread of the virus in the liver and eventually leading to impaired adaptive immunity.<sup>63</sup> The expression of interferon-stimulated genes (commonly referred to as ISGs) compromises over 300 antiviral molecules that synergistically exert innate immunity and are under control of the catalysis of retinol and retinoic acid biogenesis.<sup>65</sup> Interestingly, the toxicity of these two substances can be suppressed by *ALDH* metabolism. It means that inhibition of *ALDH* will hinder the body's antiviral ability through the ISGs pathway. Therefore, one of the molecular mechanisms for the synergism between HCV and alcohol abuse in liver disease progression is hepatocyte metabolism involving ethanol-retinol metabolic competition.<sup>66</sup>

In addition, activated T cells can be combined with other immune cells to form a positive feedback effect, being aroused by various cellular factors in turn, in a bid to stir up inflammation and inhibiting further liver damage. Gao *et al.*<sup>37</sup> discovered the phenomena that alcohol-fed *Aldh2*<sup>-/-</sup> mice were less sensitive to concanavalin A-induced T cell hepatitis than wild type mice. Their further study suggested that ACH directly restrained cytokine production in T cells by means of the inhibition of aerobic glycolysis or stimulation of corticosterone release, leading to the occurrence of suppressed T cell hepatitis in ethanol-fed *Aldh2*<sup>-/-</sup> mice. What is more, there is a certain correlation between the HBV epi-





**Fig. 3. Effect of ALDH2 in HCV infection and toxic aldehydes.** Ethanol is converted to acetaldehyde by the cytosolic enzyme ADH. Then acetaldehyde is converted to acetate by ALDH2. ACH could help to activate the expression of miR-122 and miR-34a, both of which are able to stimulate hepatitis C virus replication, leading to apoptosis. Kupffer cells and hepatic stellate cells (HSC) are driven by interleukins and aggregate to participate in the phagocytosis and clearance of apoptotic bodies. ACH also increases the activity of PP2A. PP2A could reduce methylation of signal transducer and activator of transcription (STAT)-1 and formation of the protein inhibitor of activated STAT-1 PIAS-1-STAT-1 complex. 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) are toxic aldehydes in human bodies, which are produced by reactive oxygen species (ROS). Meanwhile, ROS could activate nuclear factor-kappa B (NF-κB) and its downstream targets.

demic area and the distribution of *ALDH2* gene-defect areas in East Asia.<sup>67</sup> Almost all patients with *ALDH2* mutation genotypes are linked with HBV infection.<sup>68</sup> Among patients with chronic HBV, homozygous carriers of the *ALDH2*\*2 mutant gene have a significantly increased risk of developing liver cirrhosis.

Generally speaking, there is an optimistic link between *ALDH2* and HCV, and alcohol is undoubtedly a factor that aggravates the development of HCV disease and may also be a break-through point in the design of research experiments.

### Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer mortality in the world.<sup>69</sup> In China, HCC has emerged as one of the top three malignant tumors, according to rankings by prevalence and mortality.<sup>70,71</sup> The prevalence rates of HCC were reportedly 0.03%, 0.01%, <0.01%, and <0.01% among the general population in China, the USA, Europe, and Japan, respectively.<sup>25</sup> Meanwhile, HCC cases are increasing rapidly in China,<sup>69</sup> which accounts for approximately 90% of all cases of primary liver cancer.<sup>72</sup> Therefore, scientists have been endeavoring to explore the relationship between *ALDH2* gene mutation and HCC (Table 2<sup>35,73–79</sup>).

Generally, HBV infection and ALD are two major liver diseases with HCC developing tendency.<sup>60</sup> Hou *et al.*<sup>73</sup> inhibited aggressive behavior *in vitro* and in mice by forcing the expression of *ALDH2* in HCC cells. Recently, Liu *et al.*<sup>35</sup> explored the association between *ALDH2* polymorphisms and the risk of HCC among CHB patients, and their result showed that *ALDH2* polymorphisms has nothing to do with HCC but does protect against developing HCC through habitual alcohol drinking, which was reported in another research study as well.<sup>74</sup> Similarly, based on an analysis of 4,155 hepatitis B surface antigen seropositive participants,

there is a distinct relationship between an increased risk of HCC in the HBV-positive cirrhosis population and *ALDH2* gene polymorphisms.<sup>75</sup> Recently, a study involving both mice and human patients showed that *ALDH2* gene deficiency correlates well with a higher risk for advancement of alcohol related-fibrosis to HCC.<sup>76</sup>

A meta-analysis conducted by Chen *et al.*<sup>77</sup> found that the *ALDH2* rs671 polymorphism is not associated with HCC susceptibility in East Asians, and this is similar to the conclusion from Liu *et al.*<sup>35</sup> Interestingly, Huang *et al.*<sup>78</sup> found that the *ALDH2* polymorphisms had a certain impact on resolution of HCC in patients. The result showed that HCC patients with a defective allele of *ALDH2* have a promising postoperative outcome, after Kaplan-Meier analysis and univariate followed by multivariate Cox proportional hazard analysis indicated that the GG genotype is an independent clinical predictor for shorter time-to-distant metastasis (adjusted  $p=0.019$ ) and shorter overall survival (adjusted  $p=0.001$ ). Although the *ALDH2*\*2 mutation itself does not lead to liver cancer directly, it will reduce *ALDH2* protein levels and liver enzyme, which eventually is related to the accumulation of ACH in the blood and carcinogenic mutations. Likely, the results of animal experiments show that the mouse *ALDH2* (E487K) mutation significantly promotes the occurrence and development of mouse liver cancer.<sup>79</sup>

Unfortunately, despite a series of strong evidence supporting ethanol as an environmental risk factor for HCC, the exact pathways by which alcohol causes HCC are still under exploration. ACH has been shown to affect DNA replication and repair mechanisms. After chronic alcohol exposure, *Aldh2*-deficient animals produce a large amount of harmful oxidized mitochondrial DNA via extracellular vesicles, which can be delivered into neighbor HCC cells and subsequently activate multiple oncogenic pathways, to promote HCC development (Fig. 4).<sup>76</sup> What is more, consuming a large amount of ethanol induces microsomal ethanol metabolism by cytochrome P4502E1 (known as CYP2E1) and leads to additional production of acetaldehyde, as well as an in-

**Table 2. Recent clinical studies on the relationship between the *ALDH2* polymorphism and HCC**

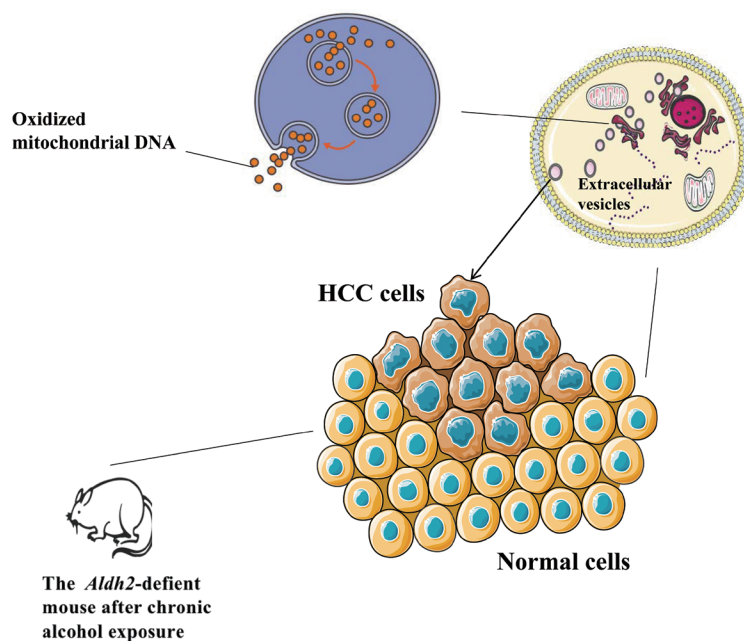
Author	Year	Conclusion	Reference
Liu <i>et al.</i>	2016	Polymorphisms in <i>ALDH2</i> had significant indirect effects on HCC risk, mediated through alcohol drinking.	35
Hou <i>et al.</i>	2017	Inhibiting aggressive behavior both <i>in vitro</i> and in mice by forcing the expression of <i>ALDH2</i> in HCC cells.	73
Ye <i>et al.</i>	2018	The mutant genotypes of <i>ALDH2</i> may be protective factors for HCC susceptibility in Guangxi Province, China.	74
Chien <i>et al.</i>	2016	GG genotype of <i>ALDH2 rs671</i> was an HCC risk predictor in cirrhotic chronic hepatitis B patients.	75
Seo <i>et al.</i>	2019	<i>ALDH2</i> deficiency is associated with an increased risk of alcohol-related HCC development from fibrosis in human patients and in mice.	76
Chen <i>et al.</i>	2020	<i>ALDH2 rs671</i> polymorphisms are not associated with HCC susceptibility in East Asians.	77
Huang <i>et al.</i>	2019	HCC patients carrying a defective allele of <i>ALDH2</i> had a favorable postoperative outcome.	78
Jin <i>et al.</i>	2015	<i>ALDH2</i> plays a role of tumor suppressor by maintaining the stability of genome in the liver, and the common human <i>ALDH2</i> variant would become an important risk factor for hepatocarcinogenesis.	79

Abbreviations: ALDH2, aldehyde dehydrogenase 2; CYP2E1, cytochrome P4502E1; HCC, hepatocellular carcinoma.

crease in free radicals that can result in cell death, DNA damage, and even production of other carcinogenic substances.<sup>80,81</sup> Other hypothesized pathways have included the transactivator protein X that is encoded by HBV and remodeled to the extracellular matrix through hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) target genes and the lysyl oxidase (HIF-1 $\alpha$ /LOX) pathway to promote HCC metastasis.<sup>82</sup> The ALDH2-acetaldehyde-redox-AMP-activated protein kinase (AMPK) axis participates in the regulation of ACH levels, which is activated by ALDH2. Therefore, identifying ALDH2 expression levels in HCC might be a useful biomarker for determining prognosis and developing targeted therapies that are urgently needed to treat patients with HCC.

In addition, human liver cancer tissue test results show

that *ALDH2*\*2 protein is extremely unstable in human liver, and the low expression of *ALDH2* protein has a certain correlation with the formation of liver cancer. The *Journal of Hepatology* also reports that a deficiency in the *ALDH2* gene expression is associated with an increased risk of HCC in patients with hepatitis B cirrhosis who overtake alcohol. Both *in vivo* and *in vitro* studies have found that liver cells from *ALDH2*-deficient mice can produce a large amount of harmful oxidized mitochondrial DNA,<sup>32</sup> which is transferred to adjacent liver cells through extracellular vesicles and can activate multiple carcinogenic pathways involving ACH (JNK, STAT3, BCL-2, and TAZ) to promote the occurrence of alcohol-related HCC.<sup>73</sup> ALDH2 could also affect metabolism by regulating the ALDH2-acetaldehyde-redox-AMPK



**Fig. 4. Effect of ALDH2 on HCC cells.** After chronic alcohol exposure, the *Aldh2*-deficient mice produce a large amount of harmful oxidized mitochondrial DNA which are delivered into neighboring hepatocellular carcinoma (HCC) cells via extracellular vesicles.

axis,<sup>83</sup> because AMPK can direct lipid metabolism to regulate tumor cell growth and survival. Actually, in proteomics studies, among the proteins related to metabolism and liver function, ALDH2 does not have the highest expression level of enzymes, compared to ALDH1, ALDH4 and ALDH9A, according to the characteristics of the metabolic subgroup;<sup>84</sup> however, we cannot look down upon the role of *ALDH2* in HCC. Zahid *et al.*<sup>85</sup> reported transcriptional suppression of alcohol metabolism regulators, and demonstrated that *ALDH2*, downstream of the mTOR signal, is partly responsible for triggering oncogenic transformation of hepatocytes, resulting in disease onset and progression in HCC *in silico*.

In summary, *ALDH2* is a potential risk factor for HCC. However, the clinical correlation between *ALDH2* gene polymorphism and the occurrence and development of liver cancer remains to be further studied.

## Conclusions

ALDH2 is a key enzyme in alcohol metabolism, and its genetic mutations are mainly clustered in East Asia. The genetic mutations of *ALDH2* will depress ALDH2 enzyme activity and provoke accumulation of ACH, which will lead to the destruction of liver cells. Importantly, *ALDH2* gene mutation and the potential impact of ACH on T cell response may become one of the factors affecting the progression of liver disease and outcomes of global liver disease. In conclusion, understanding the impact of disease progression related to the *ALDH2* gene may be helpful for the improvement of future liver disease prevention strategies.

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## Conflict of interest

The authors have no conflict of interests related to this publication.

## Author contributions

Review design (BC), drafting of the manuscript and figure design (QW), language and grammar perfection (XL), revising the manuscript for important intellectual content (QW, BC, ZZ), critical revision (ZZ).

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