

# Roles of defective *ALDH2* polymorphism on liver protection and cancer development

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**Abstract** Because serum transaminases elevate alcohol dose dependently as a consequence of liver injury, they serve as useful biological markers of excessive drinking. However, these markers are inadequate in individuals with a defective allele of the aldehyde dehydrogenase 2 gene, *ALDH2*\*2, because they show a different correlation with the amount of ethanol. For example, the serum alanine aminotransferase (ALT) level could become even lower than the baseline after alcohol intake in *ALDH2*\*2 carriers. In fact, multiple studies suggest that *ALDH2*\*2 is a hepatoprotective factor in healthy individuals. Importantly, excessive drinking is particularly dangerous in carriers of *ALDH2*\*2 because the risk of alcohol-related cancer is much higher than that for *ALDH2*\*1/\*1 carriers. Without

recognizing the genotype interaction on serum transaminase, the opportunity to warn people about potential cancer risks is missed owing to incorrect interpretation. This is particularly important in East Asian countries where approximately half of the population carries the *ALDH2*\*2 allele. To date, the mechanism of liver protection from ethanol load in individuals with *ALDH2*\*2 has not been fully elucidated. However, some reasonable mechanisms have been suggested by experimental studies, including remodelling of detoxifying systems. Further studies to uncover the whole mechanism are anticipated.

**Keywords** *ALDH2* · Gene polymorphism · Serum transaminase · Alcohol · Cancer

## Abbreviations

ALDH2	Aldehyde dehydrogenase 2
AST	Aspartate aminotransferase
ALT	Alanine transaminase
GGT	Gamma-glutamyltransferase
4HNE	4-hydroxy-2-nonenal
MDA	Malondialdehyde
SNPs	Single nucleotide polymorphisms
TNF $\alpha$	Tumour necrosis factor-alpha
GSH	Glutathione

## Introduction

A defective allele of the aldehyde dehydrogenase (*ALDH*) 2 gene, *ALDH2*\*2 (rs671), is highly prevalent in East Asian countries [1, 2]. Although the mechanism is not well understood, *ALDH2*\*2 has been reported to have positive and, surprisingly, negative associations with various kinds of diseases [3]. For example, the *ALDH2*\*2 allele is

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suggested to be a risk factor of Alzheimer's disease and osteoporosis according to epidemiological [4–6] and experimental studies [7–9]. By contrast, *ALDH2\*2* has also been reported to be a protective factor for essential hypertension [10–14] and psychiatric disorders, such as bipolar disorder [15], depression, and anxiety [16, 17].

We herein describe the function of ALDH2 and the pathophysiological impact of its defective polymorphism, *ALDH2\*2*, with particular focus on its effects on cancer development and serum transaminase levels associated with ethanol intake. We also discuss the significance of *ALDH2\*2* on the value of serum transaminases as a biological marker of excessive drinking.

## ALDH2

ALDHs catalyse the oxidation of many chemically diverse aldehydes to their corresponding acid metabolites. The ALDH superfamily plays an important role in the biotransformation of a variety of exogenous and endogenous aldehydes [18]. The human *ALDH* gene superfamily comprises 19 genes in 11 families and 4 subfamilies [19, 20].

One member of the ALDH superfamily, ALDH2, exhibits three types of enzyme activity: dehydrogenase, esterase, and reductase [21, 22]. The dehydrogenase activity requires the cofactor nicotinamide adenine dinucleotide (NAD<sup>+</sup>) and converts aldehydes to their respective carboxylic acids, e.g. acetaldehyde to acetic acid. The major lipid peroxidation products 4-hydroxy-2-nonenal (4HNE) and malondialdehyde (MDA) are also detoxified by ALDH2 [23, 24].

ALDH2 has the highest affinity for acetaldehyde, an important intermediary product of ethanol metabolism. Although acetaldehyde is also detoxified by other ALDH isozymes, including cytosolic ALDH1A1 and mitochondrial ALDH1B1, ALDH2 is the primary ALDH responsible for the clearance of ethanol-derived acetaldehyde [3, 18, 25].

## *ALDH2* polymorphism

Based on the National Center for Biotechnology Information database, nearly 300 single nucleotide polymorphisms (SNPs) have been reported for *ALDH2*. Of these, approximately 180 mutations are associated with amino acid replacement. However, r671 is the only SNP resulting in a powerful phenotypic change due to compromised protein function. The mutation site is located at exon 12, nucleotide position 42421 (transition of G to A), resulting in a Glu504 substitution to Lys. Glu504Lys in the precursor

protein is also described as Glu487Lys in the mature protein form. The allele with this mutation is called *ALDH2\*2*, in contrast to the wild type *ALDH2\*1*.

The Glu487Lys mutation severely decreases ALDH enzymatic activity with respect to all three of its enzymatic functions (as a dehydrogenase, esterase, and reductase) [22, 26]. It is reported that this alteration in ALDH2 increases the *K<sub>m</sub>* value (the concentration at which the reaction rate is half the maximum rate) for NAD<sup>+</sup> (the cofactor of ALDH2) by 150-fold compared to the wild type enzyme [27]. In fact, an ethanol challenge of Chinese volunteers was shown to elicit a 20-fold higher blood acetaldehyde level in the *ALDH2\*1/\*2* subjects than in *ALDH2\*1/\*1* subjects [28].

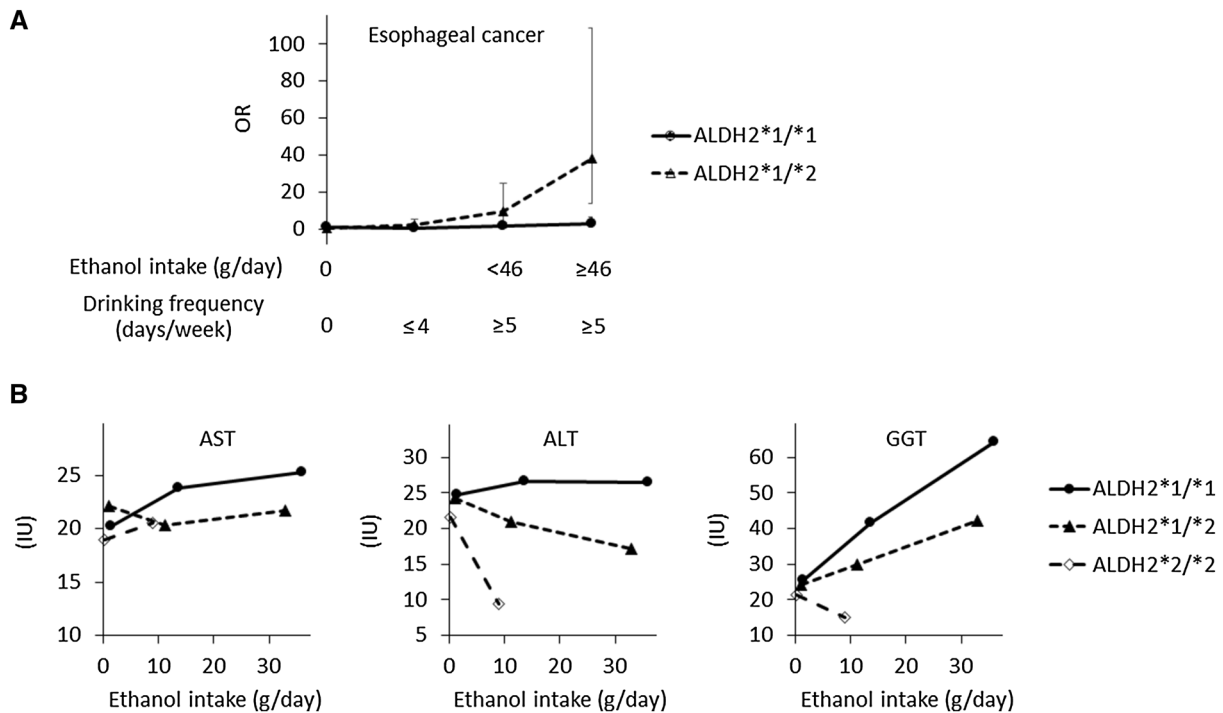
## Effect of *ALDH2\*2* on drinking behaviour

The *ALDH2\*2* polymorphism strongly inhibits drinking behaviour [10, 29]. According to Peng et al. [28], ethanol administration caused a 50 % increase in heart rate and 'terrible feelings' in *ALDH2\*1/\*2* subjects, commonly referred to as "flushing syndrome", whereas no change was observed in *ALDH2\*1/\*1* subjects. Given such vulnerability to drinking alcohol, it is not surprising that individuals with the *ALDH2\*2* allele tend not to be habitual drinkers.

However, the reinforcing properties of ethanol could overwhelm the uncomfortable symptoms of acetaldehyde; indeed, carriers of *ALDH2\*2* do still drink ethanol to a certain extent. For example, Nakamura et al. [30] reported the mean number of drinks per day of  $2.4 \pm 1.7$  in *ALDH2\*1/\*1* subjects,  $1.7 \pm 2.0$  in *ALDH2\*1/\*2* subjects, and  $0.1 \pm 0.4$  in *ALDH2\*2/\*2* subjects. Furthermore, alcoholics who carry *ALDH2\*1/\*2* were reported to consume a comparable amount of ethanol to alcoholics with *ALDH2\*1/\*1* [31].

## Effect of *ALDH2\*2* on cancerous diseases

Accumulating evidence suggests that a drinking habit for an individual with the *ALDH2\*2* allele increases the risk of cancerous diseases, including upper aerodigestive, gastric, colorectal, hepatic, and lung cancer [32–47]. For example, it was reported that individuals with the *ALDH2\*1/\*2* genotype who were heavy drinkers ( $\geq 46$  g ethanol/day and  $\geq 5$  days/week) had a 38-fold greater risk of developing oesophageal cancer compared to non-drinking individuals with the *ALDH2\*1/\*1* genotype, while drinkers with the *ALDH2\*1/\*1* allele had only a 2-fold greater risk [47]. No increased risk was found in non-drinking *ALDH2\*1/\*2* subjects (OR = 0.55, *p* = 0.226) (Fig. 1a).



**Fig. 1** Interaction between *ALDH2* polymorphism and alcohol intake on oesophageal cancer risk and indicators of excessive drinking. **a** Estimated odds ratios (OR) for oesophageal cancer with adjustment for cumulative smoking level reported by Koyanagi and colleagues (a Japanese case–control study) [47]. The error bars represent 95 %

confidence intervals. **b** Indicators of excessive drinking reported by Takeshita and colleagues (population-based study for Japanese workers) [57]. Data represent mean value. *AST* aspartate transaminase, *ALT* alanine transaminase

It is well established that DNA adduct formation has an aetiological role in cancer [48]. We previously reported that acetaldehyde-derived DNA adducts are elevated to a greater extent in the liver and stomach of *Aldh2* knockout mice following ethanol administration than in the liver and stomach of wild type mice [49, 50]. Similarly, Ishikawa and colleagues [51] showed that subjects carrying the *ALDH2\*2* allele who consumed ethanol more than three times per week had greater DNA damage, as reflected by an increased micronuclei frequency. Furthermore, cytochrome P450 (*CYP2E1*), which is known to form reactive oxygen species [52] and enhance pro-carcinogen activation [53], is upregulated in *Aldh2* knockout mice, and is further induced by ethanol administration [54]. As such, *ALDH2\*2* has the potential to promote the risk of cancers induced by alcohol consumption (Fig. 1).

**Effect of *ALDH2\*2* on indicators of excessive drinking**

Serum alanine transaminase (ALT), aspartate aminotransaminase (AST), and gamma-glutamyltransferase (GGT) levels are generally used as biological markers of excessive drinking, which elevate dose dependently as a

result of alcoholic liver injury [55, 56]. These markers have been presumed to be particularly high in drinkers that carry *ALDH2\*2*, because this allele is expected to promote alcoholic liver injury. However, in reality, the opposite results have been obtained in multiple epidemiological and experimental studies.

Takeshita and colleagues [57] studied Japanese male workers who were free from hepatic viruses and from high transaminase activities (<100 IU/L), and showed that subjects with *ALDH2\*2* consuming moderate to high levels of alcohol had lower serum AST, ALT, and GGT levels (e.g. 25, 27, and 64 IU/L, respectively, in subjects with *ALDH2\*1\*1* versus 22, 17, and 42 IU/L, respectively, in subjects with *ALDH2\*1\*2*) (Fig. 1b). Another study on Japanese male workers suggested that *ALDH2\*2* is an independent protective factor against elevation of the serum ALT level (odds ratio of 0.16) from multivariate logistic regression analysis, including body mass index and drinking habits in the model [58]. This finding was confirmed in a subsequent large-scale epidemiological study [10].

Furthermore, animal experiments using *Aldh2* knockout mice showed the same tendency; serum ALT decreased after 5 weeks of simple ethanol loading (without any additional treatments to induce liver injury) in *Aldh2*

knockout mice, while no change was observed in wild type mice [59]. In a subsequent study, the decrease of ALT level in *Aldh2* knockout mice was reported to be sustained for as long as the ethanol loading was administered, whereas wild type mice showed elevation of serum ALT [60]. Moreover, a liver injury model induced with a high-fat diet and carbon tetrachloride also showed lower serum ALT values in *Aldh2* knockout mice than in wild type mice [61].

### Mechanism of liver damage amelioration induced by congenital lack of ALDH2

Some mechanisms have been suggested to explain these observed interactions between the *ALDH2* genotype and drinking on serum transaminase levels (Fig. 2). For example, the effects of *ALDH2\*2* on curbing acetaldehyde metabolism could produce benefits. Cyanamide, an ALDH inhibitor, diminished the ethanol-elicited increase of oxidative stress by inhibition of reduced nicotinamide adenine dinucleotide (NADH) formation in the mitochondria during the metabolism of acetaldehyde to acetate [62, 63]. This theory suggests that the *ALDH2\*2* allele can ameliorate the sharp increase of NADH that causes mitochondrial dysfunction, resulting in oxidative stress and hepatocyte damage from alcohol consumption. Supporting this hypothesis, *Aldh2* knockout mice showed lower MDA and higher glutathione (GSH) levels after a single-dose administration of ethanol [64].

Ethanol loading to people with *ALDH2\*2* might decrease tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) through inhibition of the associated transcription pathways (nuclear factor- $\kappa$ B pathway and mitogen-activated protein kinase pathway, which are considered to be important pathways for alcoholic liver injury [65–67]) [59, 68]. An

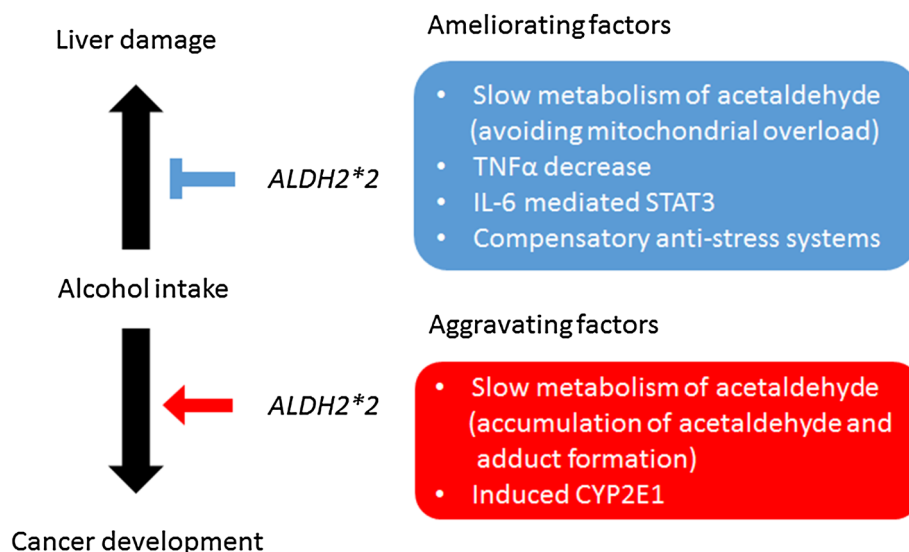
*in vivo* experiment using *Aldh2* knockout mice showed decreased serum ALT, TNF $\alpha$ , and extracellular signal-regulated kinase 2 levels after 5 weeks of ethanol administration, and significant correlations were observed among these three factors [59].

A different mechanism was suggested using liver injury models, in which interleukin-6-induced signal transducer and activator of transcription 3 relieve hepatic steatosis and result in lower serum ALT levels [61]. Supporting this finding, Yokoyama and colleagues [69] performed an epidemiological study on Japanese alcoholic men and reported that the serum triglyceride concentration was lower in *ALDH2\*1/\*2* carriers than in *ALDH2\*1/\*1* carriers (the least squares means adjusted for age and drinking amount were 103 and 113 mg/dL, respectively).

Another convincing explanation for these associations involves remodelling of stress response systems that are induced in a compensatory manner to mitigate the cytotoxicity of endogenous aldehydes. This is because individuals lacking ALDH2 will likely be exposed to higher levels of endogenous aldehydes from birth, such as 4HNE and MDA [23, 24], thereby requiring an alternate defence system. In fact, *Aldh2* knockout mice had higher levels of heme oxidase 1, an essential defensive factor against cellular oxidative stress, without any pre-treatment [54]. Furthermore, it was reported that a series of GSH-producing enzymes was upregulated in *ALDH2\*2* targeting mice [70]. A higher GSH level in individuals with *ALDH2\*2* has been confirmed in humans according to a case–control study of children in China [71]. In this study, better clinical outcomes were reported for *ALDH2\*2* carriers, such as lower serum troponin level and shorter stay period in intensive care unit [71].

The compensatory protection system may protect *ALDH2\*2* carriers not only from alcoholic liver damage

**Fig. 2** Proposed mechanism of amelioration of liver damage and aggravation of cancer development by a combination of alcohol intake and possession of a defective polymorphism of *ALDH2* (*ALDH2\*2*). *ALDH2* aldehyde dehydrogenase 2, *TNF $\alpha$*  tumour necrosis factor- $\alpha$ , *STAT3* signal transducer and activator of transcription 3



but also from other pathologies, including cancerous diseases. To the best of our knowledge, *ALDH2*\*2 has not been reported as a cancerous risk factor for non-drinkers, in spite of the decreased metabolic ability for 4HNE, which is a well-known cancer initiator [72]. Interestingly, a recent epidemiological study in Taiwan demonstrated that *ALDH2*\*2 was a protective factor of hepatocellular carcinoma (HCC) for patients with hepatitis B-positive cirrhosis [73]. In this study, HCC did not appear to be induced by alcohol; only 14 % of the subjects had a drinking habit and no association between drinking and HCC was found. The multivariate-adjusted hazard ratio of *ALDH2*\*1/\*2 for HCC development was calculated to be 0.35 when the *ALDH2*\*1/\*1 genotype was set as reference. This finding suggests that *ALDH2*\*2 could be a protective factor for alcohol-independent HCC. Induction of anti-oxidative proteins might be one of the defence systems for HCC development. Another possible mechanism could be that possession of *ALDH2*\*2 ameliorates PI3K/Akt pathway activation [74]. It is known that the PI3K/Akt pathway is hyper-activated in tumours, resulting in the promotion of malignant growth [75].

Such amelioration of liver damage conferred by *ALDH2*\*2 may be limited to the type of injury. For example, the liver injury model mentioned above suggested protective effects such as inhibition of fatty degeneration; nevertheless, adverse effects were also reported, such as aggravation of the inflammatory response and fibrosis [61]. Simple ethanol administration does not cause such a marked discrepancy; however, hepatocyte ballooning was observed only in *Aldh2* knockout mice in spite of their lower TNF $\alpha$  and serum ALT levels [59]. However, in practice, people who carry *ALDH2*\*2 drink much less than the amount given to experimental animals (see “[Effect of \*ALDH2\*\\*2 on drinking behaviour](#)”). This may explain why epidemiological studies have only observed the advantageous aspect of *ALDH2*\*2 for liver tissue function.

### Conclusion: significance of the interaction between *ALDH2* polymorphism and alcohol intake

Serum transaminase is a useful indicator of excessive drinking for self-monitoring by drinkers themselves and for their surrounding healthcare workers [55, 56]. However, it is less useful in people with *ALDH2*\*2, because it does not elevate dose dependently with ethanol in the same manner as observed in people with *ALDH2*\*1/\*1 (see [Effect of \*ALDH2\*\\*2 on indicators of excessive drinking](#)). As a result, the opportunity to warn people with *ALDH2*\*2 of excessive drinking for the prevention of alcohol-related diseases, particularly cancerous diseases, could be lost when relying on only serum transaminase levels as a marker.

Furthermore, individuals with *ALDH2*\*2 have higher risks of cancer development (see [Effect of \*ALDH2\*\\*2 on cancerous diseases](#)). In other words, *ALDH2*\*2 carriers need to be more strict about limiting or refraining from drinking than *ALDH2*\*1/\*1 carriers, and serum transaminase is an inadequate indicator of excessive drinking for carriers of *ALDH2*\*2. Thus, effective risk assessment and disease prevention require screening for *ALDH2* genotype.

The influence of the *ALDH2*\*2 allele on the value of serum transaminases and GGT as markers of excessive drinking could be modified in individuals with additional pathological conditions such as metabolic disorders of lipids and sugars. It would be useful to investigate this issue further in the future. Moreover, the interaction of *ALDH2* polymorphism and alcohol intake on other alcohol-related diseases such as cardiovascular diseases and reproductive disorders should be well studied.

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### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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