[Frontiers in Bioscience E2, 1344-1354, June 1, 2010]

Effects of acetaldehyde inhalation in mitochondrial aldehyde dehydrogenase deficient mice (*Aldh2*$^{-/-}$)

Tsunehiro Oyama[1], Haruna Nagayoshi[2,3], Tomonari Matsuda[2], Megumi Oka[2], Toyohi Isse[4], Hsu-Sheng Yu[1], Thi-Thu-Phuong Pham[1], Masayuki Tanaka[1], Norio Kagawa[5], Kazuhiro Kaneko[6], Toshihiro Kawamoto[1]

[1]Departments of Environmental Health, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, 807-8555, Japan, [2]Research Center for Environmental Quality Management, Kyoto University, Otsu, Shiga, 520-0811, Japan, [3]Osaka Prefectural Institute of Public Health, Osaka, 537-0025, Japan, [4]Section of Postgraduate Guidance, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, 807-8555, Japan, [5]Global COE, Nagoya University School of Medicine, Nagoya, Aichi, 466-8550, Japan,[6]Division of Digestive Endoscopy/Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, 277-8577, Japan

TABLE OF CONTENTS

1. ABSTRACT

Human body might be exposed to acetaldehyde from smoking or occupational environment, which is known to be associated with cancer through the formation of DNA adducts, in particular, N^2-ethylidene-2'-deoxyguanosine (N^2-ethylidene-dG). Aldehyde dehydrogenase 2 (ALDH2) is the major enzyme that contribute to the detoxification of acetaldehyde in human body. In this study, wild type (*Aldh2*$^{+/+}$) and *Aldh2*KO (*Aldh2*$^{-/-}$) mice were exposed to the air containing 0, 125, 500 ppm acetaldehyde for 2 weeks. After inhalation, levels of N^2-ethylidene-dG in the chromosomal DNA were analyzed by liquid chromatography tandem mass spectrometry (LC/MS/MS). N^2-ethylidene-dG levels in livers of *Aldh2*$^{-/-}$ mice were always lower than those of *Aldh2*$^{+/+}$ mice, suggesting that *Aldh2* deficiency might cause the induction of acetaldehyde metabolizing enzymes in the liver such as P450s. The differences between *Aldh2*$^{-/-}$ and *Aldh2*$^{+/+}$ mice were greater in the order of nasal epithelium > lung > dorsal skin, suggesting that nasal epithelium and lung are the major target sites for acetaldehyde. Acetaldehyde inhalation may cause a high risk in nasal epithelium and lung cancers for individuals with inactive ALDH2.

2. INTRODUCTION

Alcohol misuse is linked to a variety of social and medical problems. The number of Japanese alcoholism patients was about 2.5 million in 1995 and has been gradually increasing (1, 2). Alcohol misuse affects many organs, and is associated with the incidence of various cancers, such as esophageal cancer (3-5). Epidemiological evidence indicates that alcohol consumption is related to the development of various cancers and liver diseases, all of which are associated with altered levels of various intracellular oxidizing enzymes (6-8). Therefore, the metabolic pathway of ethanol and its variants among individuals are of great interest for risk assessment and prevention of diseases caused by alcohol abuse.

Ingested ethanol is oxidized by cytosolic class I alcohol dehydrogenase 2 (ADH2) to acetaldehyde, which is subsequently oxidized by mitochondrial aldehyde dehydrogenase 2 (ALDH2) to produce non-toxic acetate (9, 10). Human ALDH isozymes are divided into two groups determined by their Michaelis constant values for acetaldehyde; the low K_m ALDH (ALDH1 and ALDH2), and high K_m ALDH (ALDH3 and ALDH4). The K_m values of ALDH3 and ALDH4 are in millimolar (5–83 mM) (11),

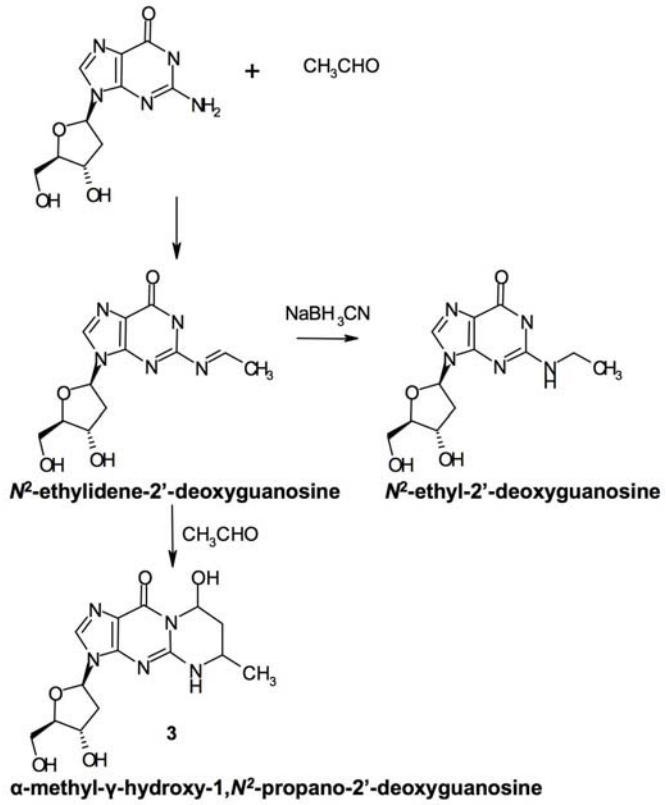

Figure 1. Formation of acetaldehyde-dG adducts. The reaction of deoxyguanosine with acetaldehyde produces N^2-ethylidene-2'-deoxyguanosine. The product further reacts with acetaldehyde and produces α-methyl-γ-hydroxy-1,N^2-propano-2'-deoxyguanosine. To determine the amount of N^2-ethylidene-2'-deoxyguanosine (N^2-ethylidene-dG), its reduced product, N^2-ethyl-2'-deoxyguanosine (N^2-Et-dG), is analyzed by LC/MS/MS as described in "Materials and Methods".

cytosolic ALDH1 in micromolar (180 μM), and mitochondrial ALDH2is in nanomolar (200nM) (12), suggesting that ALDH2 is a key enzyme responsible for catalyzing oxidation acetaldehyde in human liver.

*ALDH2*2* isa genetic polymorphism of ALDH2 resulting in an amino acid substitution from glutamic acid at 487 to lysine (E487K), and is particularly prevalent in Asian populations (13). ALDH2 functions as a homotetramer, and the inactive subunit produced by the *ALDH2*2* allele acts in a dominant negative fashion. It is predicted that individuals who possess the *ALDH2*1/2*2* genotype will have only 6.25% of the normal ALDH2 protein and that other tetramers containing one or more of the ALDH2*2 subunits are mostly inactive. However, when taken together, the overall measured activity of the five possible tetramer combinations of the *ALDH2*1/2*2* genotype is approximately 13% (14). On the other hand, individuals who are *ALDH2*2/2*2* homozygous have no ALDH2 activity.

Individuals with *ALDH2*2* allele show high blood acetaldehyde concentrations after intake of only a moderate amount of alcohol (2). Acetaldehyde itself is a carcinogen that induces nasal tumors in experimental animals by inhalation (15), and is thought to be a tumor-initiator because of its mutagenic and DNA-damaging properties (16-19). It has been confirmed that acetaldehyde associated with alcoholic beverages is carcinogenic to human (Group I) (20). As a consequence of the decreased acetaldehyde metabolism, the *ALDH2*2* allele is associated with alcohol-induced flushing, and is also positively related to hepatocellular carcinoma (21, 22), oral cancer and esophageal cancer (5), while it negatively affects coronary heart disease (2, 23).

Recently, an analytical method was developed for the quantitative determination of acetaldehyde-derived stable DNA adducts, N^2-ethyl-2'-deoxyguanosine (N^2-Et-dG), α-*S*- and α-*R*-methyl-γ-hydroxy-1,N^2-propano-2'-deoxyguanosine (α-*S*-Me-γ-OH-PdG and α-*R*-Me-γ-OH-PdG) using sensitive liquid chromatography tandem mass spectrometry (LC/MS/MS) (24, 25). Other than these stable DNA adducts, the reaction of acetaldehyde with dG results in the formation of an unstable Schiff base at the N^2 position of dG (N^2-ethylidene-dG) (Figure 1). Wang *et al*. showed that N^2-ethylidene-dG in human liver DNA is relatively stable and the presence of this adduct could be confirmed by detection of N^2-Et-dG after reduction of DNA during isolation and enzymatic hydrolysis (26). They showed that when the reduction step was included during these steps, a few 100 times more N^2-Et-dG was detected in some cases.

Aldh2 knockout (KO) mice have already been generated in our laboratory (2, 27). These mice (C57BL/6) lacking *Aldh2* should be a useful animal model to investigate the effects of Aldh2 deficiency (2). Since susceptibility to inhalation toxicity of acetaldehyde is obscure in individuals with the *ALDH2*2* allele, we evaluated the production of N^2-ethylidene-2'-deoxyguanosine DNA adducts as acetaldehyde-derived DNA adducts in target organs of *Aldh2* KO mice treated with acetaldehyde inhalation.

3. MATERIALS AND METHODS

3.1. Wild type (*Aldh2*$^{+/+}$ mice) and *Aldh2*KO mice (*Aldh2*$^{-/-}$ mice)

Male C57BL/6 (*Aldh2*$^{+/+}$) mice, at 10 weeks of age, were purchased from Charles River Japan, Inc. (Yokohama) and male *Aldh2* KO (*Aldh2*$^{-/-}$) mice, at 10 weeks of age, were generated as previously described (27). *Aldh2*$^{-/-}$ mice were back-crossed with C57BL/6 strain for more than 10 generations. These mice were housed in specific pathogen-free units of the Division of Animal Care at the University of Occupational and Environmental Health. Seven or ten mice were placed in polycarbonate cage (W215xH140xD320 mm). Mice were adjusted to the new environment for a week before use. Autoclaved cages, floor beds, and rodent chow were used. The mice cage was cleaned every day. All the mice were treated in accordance with the guidelines of the Animal Welfare and approved by the Ethics Committee of the Animal Care and Experimentation of the UOEH (28-30).

3.2. Treatments

A group of 10 mice of each *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ were exposed to filtered atmospheric air (0 ppm) and served as controls. A second group of seven of each *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were exposed to air containing 125 ppm acetaldehyde (125 ppm exposed-group). A third group of ten of each *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were exposed to air containing 500 ppm acetaldehyde (500 ppm exposed-group). Mice were divided randomly and Acetaldehyde levels were evaluated in the cage every 6 hours by acetaldehyde detector tubes (Gastec corp., Kanagawa, Japan) and Sep-Pak DNPH-Silica (Waters corp., MA, USA) (29). Mice were exposed to atmospheres containing acetaldehyde for 24 h/day during 14 days. During this period body weights were recorded every day and the mice were sacrificed at the end of the experiment..

3.3. Blood acetaldehyde concentration

Mouse blood was collected from the decapitated trunk into liquid nitrogen-cooled plastic tubes and stored. The blood (0.5mL) was transferred into ice cold 0.6N perchloric acid solution (PCA) and centrifuged. Acetaldehyde concentration was measured as previously described (28, 31), using Hewlett-Packard headspace sampler (HP7694; Wilmington, DE) andHewlett-Packard gas chromatograph (HP6890, Wilmington, DE) equipped with a 60 m x 0.25 mm inner diameter AQUATIC capillary column (GL Sciences, Tokyo, Japan) with a film thickness of 1.0 μm that was connected to a mass spectrometer (JOEL JMS-BU20, Tokyo, Japan).

3.4. DNA isolation from mouse organs

For quantification of N^2-Et-dG, DNA was isolated from mouse organs using Gentra Puregen tissue kit as described previously (24). The procedure was basically carried out as per manufacture's instructions except the addition of $NaBH_3CN$ to all solutions (final concentration: 100 mM). After purification, DNA was dissolved in 10 mM Tris-HCl/5 mM EDTA buffer (pH 7.0), extracted with chloroform and precipitated with ethanol.

3.5. DNA digestion (25)

20 μg aliquots of DNA were digested into their constituent 2'-deoxyribonucleoside-3'-monophosphate units by adding15μl of 17 mM citrate plus 8 mM $CaCl_2$ buffer that contained micrococcal nuclease (22.5 U) and spleen phosphodiesterase (0.075 U) plus internal standards. The solutions were mixed and incubated for 3 h at 37 °C, after which alkaline phosphatase (1 U), 10μl of 0.5 M Tris-HCl (pH 8.5), 5μl of 20 mM $ZnSO_4$ and 67μl of distilled water were added and incubated for further 3 h at 37 °C. The digested sample was extracted twice with methanol. The methanol fractions were evaporated to dryness, resuspended in 50 μl of distilled water and subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS).

3.6. Instrumentation (25)

LC/MS/MS analyses were performed using a Shimadzu LC system (Shimadzu, Kyoto, Japan) interfaced with a Quattro Ultima triple stage quadrupole MS (Waters-Micromass, Manchester, UK). The LC column was eluted over a gradient that began at a ratio of 5% methanol to 95% water and was changed to 40% methanol over a period of 30 min, changed to 80% methanol from 30 to 35 min, and finally returned to the original starting conditions, methanol: H_2O = 5:95, for the remaining 11 min. The total run time was 46 min. Sample injection volumes of 20 μl each were separated on a Shim-pack XR-ODS column (3.0 mm×75 mm, 2.2 μm) and eluted at a flow rate of 0.2 ml/min. Mass spectral analyses were carried out in positive ion mode with nitrogen as the nebulizing gas. The ion source temperature was 130 °C, the desolvation gas temperature was 380 °C and the cone voltage was operated at a constant 35 V. Nitrogen gas was also used as the desolvation gas (700 l/h) and cone gas (35 l/h), and argon was used to provide a collision cell pressure of 1.5×10^{-3} mbar. Positive ions were acquired in multiple reactions monitoring (MRM) mode. The MRM transitions monitored were as follows: [$^{15}N_5$]8-oxo-dG, *m/z* 288 → 172; 8-oxo-dG, *m/z* 283 → 167; [$^{15}N_5$] N^2-Et-dG, *m/z* 301→ 185 and N^2-Et-dG, *m/z* 295.5 → 179.9. The amount of each adduct was quantified by the ratio of the peak area of the target adducts to that of its stable isotope. Quanlynx (version 4.0) software (Waters-Micromass) was used to create standard curves and to calculate adduct concentrations. The amount of deoxyguanosine was monitored at 254 nm by a Shimadzu SPD-10A UV-Visible detector that was in place before the tandem MS.

3.7. Statistics

Analysis of co-variance was carried out on body weights. For changes of DNA adduct levels, the chi-square test was used.

Table 1. The mean mice body weights of the various groups. These data from the previous report (31)

		Aldh2$^{+/+}$ mice			*Aldh*$^{-/-}$ mice		
Body weight (g)		n	mean	SD	n	Mean	SD
Control group	Before treatment	10	27.1	1.25	10	26.5	1.66
	After treatment	10	28.2	1.37	10	27.9	1.73
125 ppm exposure group[1]	Before treatment	7	27.8	1.33	7	27.4	1.61
	After treatment	7	27.4	1.32	7	27.7	1.75
500 ppm exposure group[2]	Before treatment	10	26.6	1.79	10	27.1	1.41
	After treatment[3]	10	21.8	1.21	10	23.9	1.23

[1]Exposed to atmospheres containing acetaldehyde at levels of 125 ppm, [2]Exposed to atmospheres containing acetaldehyde at levels of 500 ppm, [3]Comparing with control group after treatment and 500 ppm exposure group before treatment ($p<0.01$)

4. RESULTS

4.1. Blood acetaldehyde concentration and body weight from the previous report (31).

The average actual concentration of 125 ppm exposure groups was 126.3 ppm, and the actual exposure of 500 ppm groups was 510.5 ppm. The mean blood acetaldehyde concentration of *Aldh2*$^{+/+}$ mice (n=3) and *Aldh2*$^{-/-}$ mice (n=3) in the 125 ppm exposed-groups were 1.65 μM and 2.39 μM, respectively. Those of *Aldh2*$^{+/+}$ mice (n=3) and *Aldh2*$^{-/-}$ mice (n=3) in the 500 ppm exposed-groups were 1.72 μM and 8.90 μM, respectively. The mean blood acetaldehyde concentration of *Aldh2*$^{-/-}$ mice was more than five times as high as that of *Aldh2*$^{+/+}$ mice in the 500 ppm exposed-groups. As shown in Table 1, the mean body weight of 500 ppm exposed-groups was significantly reduced after treatment, although control and 125 ppm exposed-groups did not show any visible weight loss ($p < 0.01$).

4.2. DNA adduct levels of target organs in mice treated with acetaldehyde inhalation

To determine the level of N^2-ethylidene-dG in chromosomal DNA, tissue samples were homogenized in lysis buffer containing the strong reducing agent $NaBH_3CN$, followed by DNA purification in the presence of $NaBH_3CN$. During the purification step, N^2-ethylidene-dG is reduced and converted to stable N^2-Et-dG that can be sensitively quantified (24). Typical LC/MS/MS-chromatograms of N^2-Et-dG derived from N^2-ethylidene-dG in nasal epithelium DNA of 125 ppm exposure groups were shown in Figure 2. The N^2-ethylidene-dG levels of nasal epithelium DNA from *Aldh2*$^{-/-}$ mice was more than eight times higher than that observed in *Aldh2*$^{+/+}$ mice exposed to 125 ppm.

In the liver, the average N^2-ethylidene-dG levels of DNA from *Aldh2*$^{+/+}$ mice (n=10) and *Aldh2*$^{-/-}$ mice (n=10) of 0 ppm groups were 62.4 ± 31.0 and 40.6 ± 26.7 adducts per 108 bases, respectively. In the 125 ppm group, the N2-ethylidene-dG levels from Aldh2+/+ mice (n=7) and Aldh2-/- mice (n=7) increased to 87.7 ± 35.0 and 69.0 ± 49.9 adducts per 108 bases, respectively. Those of Aldh2+/+ (n=10) and Aldh2-/- mice (n=10) exposed to 500 ppm further increased to 90.1 ± 36.0 and 84.6 ± 67.2 adducts per 108 bases, respectively (Figure 3). Intriguingly, the liver DNA of Aldh2-/- mice always showed decreased levels of N2-ethylidene-dG compared with that of Aldh2+/+ mice, although both Aldh2$^{+/+}$ and *Aldh2*$^{-/-}$ mice showed increased levels of N^2-ethylidene-dG along with the increase of acetaldehyde concentrations.

In the nasal epithelium of 125 ppm groups, the N^2-ethylidene-dG levels were much higher than in other organs of both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice. In the nasal epithelium, the N^2-ethylidene-dG level of *Aldh2*$^{-/-}$ mice (n=3, 581 ± 61.4) was greatly increased compared with *Aldh2*$^{+/+}$ mice (n=3, 193 ± 158) (Figure 4).

In the lung of 125 ppm groups, *Aldh2*$^{-/-}$ mice showed a higher level of N^2-ethylidene-dG (n=7, 68.4 ± 12.4) than *Aldh2*$^{+/+}$ mice (n=7, 54.7 ± 8.39) as shown in Figure 5;this difference is statistically significant. In 500 ppm groups, the difference between *Aldh2*$^{-/-}$ (n=10, 283 ± 82.3) and *Aldh2*$^{+/+}$ mice (n=10, 171 ± 49.8) was greater than that observed in the 125 ppm groups. In the lung, dramatically increased levels of N^2-ethylidene-dG were observed in the 500 ppm groups only, in both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice although it occurred in the 125 ppm groups as well in the nasal epithelium.

Compared to other organs, the level of N^2-ethylidene-dG was low in the control group in the dorsal skin of both *Aldh2*$^{+/+}$ (n=10, 26.5 ± 3.9) and *Aldh2*$^{-/-}$ mice (n=10, 28.0 ± 4.8). in the 500 ppm exposed-group, the value was increased approximately 4-fold compared with control group in *Aldh2*$^{-/-}$ mice (n=10, 122 ± 52.4) while it was doubled in *Aldh2*$^{+/+}$ mice (n=10, 48.6 ± 16.8) (Figure 6).

5. DISCUSSION

Previously, Isse *et al.* showed that the blood acetaldehyde concentration of *Aldh2*$^{-/-}$ mice (247.2 μM) was greatly higher than that of the *Aldh2*$^{+/+}$ mice (14.0 μM) one hour after the administration of ethanol by gavage at doses of 5.0 g/kg body weight (28). Matsuda *et al.* showed that in the liver of alcohol fed mice (20% ethanol for 5 weeks) the adduct level of *Aldh2*$^{-/-}$ mice (79.9 ± 14.2 adducts per 10^7 bases) was much higher than *Aldh2*$^{+/+}$ (7.9 ± 1.8 adducts per 10^7 bases), and indicated that the N^2-ethylidene-dG level in the liver was alcohol- and *Aldh2* genotype-dependent (24). In our inhalation experiments, there were no differences in the levels of N^2-ethylidene-dG in the liver between *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice (Figure 3), although the mean blood acetaldehyde concentration of *Aldh2*$^{-/-}$ mice was more than five times as high as that of *Aldh2*$^{+/+}$ mice in the 500 ppm exposure groups. We also observed that the adduct level of *Aldh2*$^{-/-}$ mice was rather low in the liver compared to *Aldh2*$^{+/+}$ mice, although the adduct level of *Aldh2*$^{-/-}$ mice was always higher in other organs than that of *Aldh2*$^{+/+}$ mice. Acetaldehyde is metabolized, not only by ADH2-ALDH2 metabolic

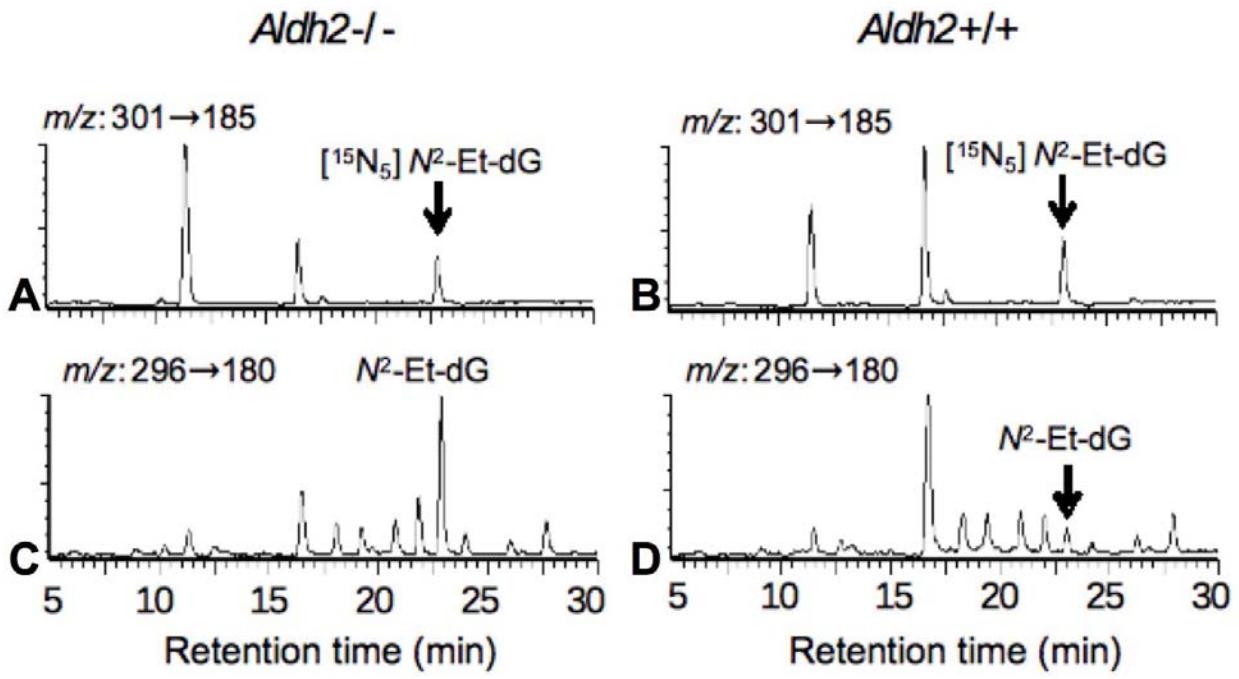

Figure 2. Representative chromatograms of the N^2-Et-dG analysis by LC/MS/MS. A and B, representative LC/MS/MS chromatograms of transition *m/z* 301→185 for [U-$^{15}N_5$] N^2-Et-dG as an internal standard (24). C and D: Typical LC/MS/MS chromatograms of N^2-ethyl-2'-deoxyguanosine (N^2-Et-dG) derived from N^2-ethylidene-2'-deoxyguanosine (N^2-ethylidene-dG) in nasal epithelium DNA of *Aldh2*$^{-/-}$ (C) and *Aldh2*$^{+/+}$ (D) mice in 125 ppm exposure groups.

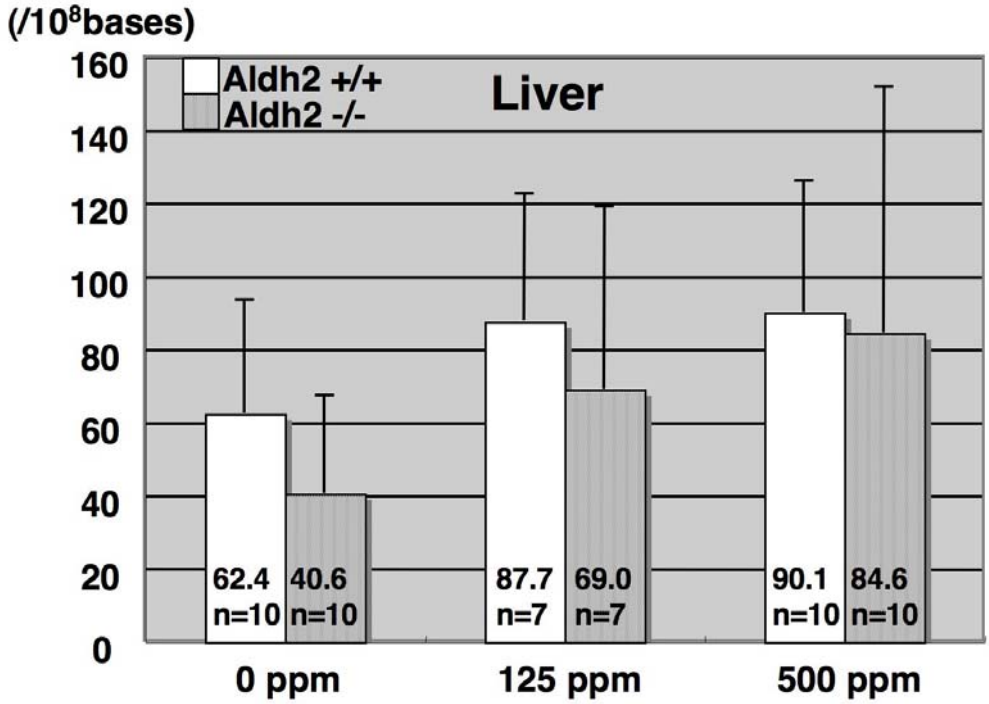

Figure 3. The N^2-ethylidene-dG level in the liver DNA . The levels of N^2-ethylidene-dG in the liver chromosomal DNA from both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were analyzed.

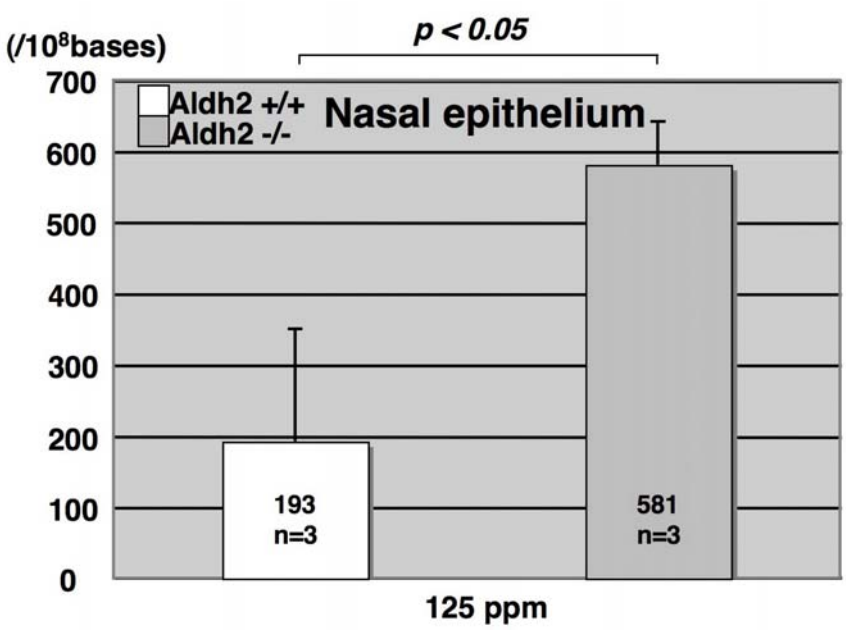

Figure 4. The N^2-ethylidene-dG level in the nasal epithelium DNA . Nasal epithelium tissues were dissected from nasal cavity, using a spatula. The levels of N^2-ethylidene-dG in the nasal epithelium chromosomal DNA from both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were analyzed.

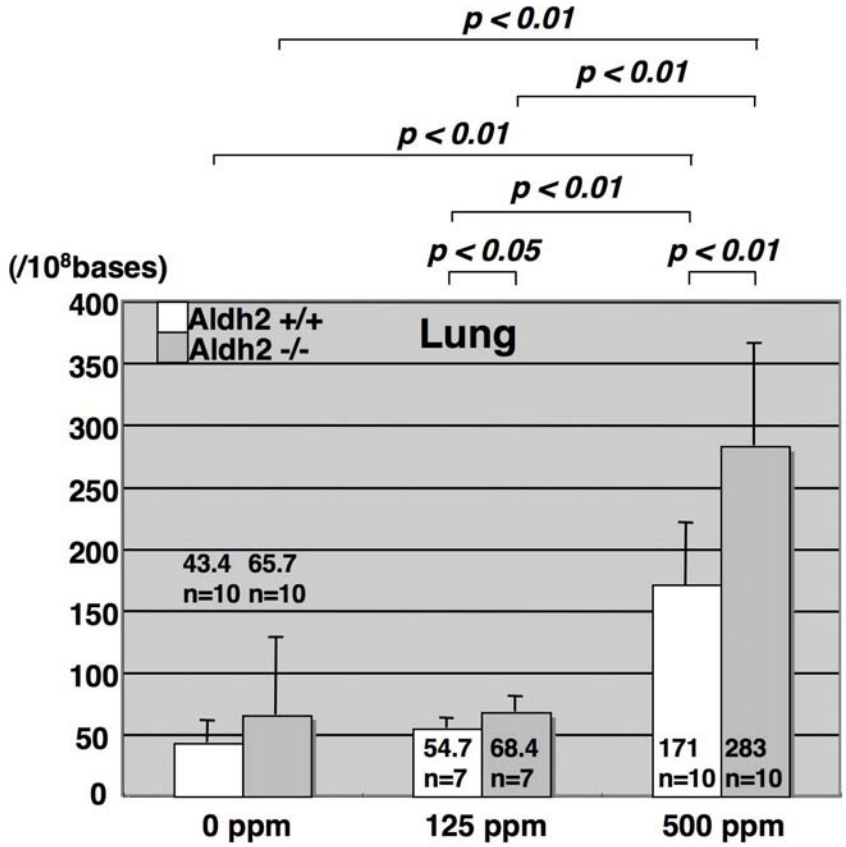

Figure 5. The N^2-ethylidene-dG level in the lung DNA . The levels of N^2-ethylidene-dG in the lung chromosomal DNA from both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were analyzed.

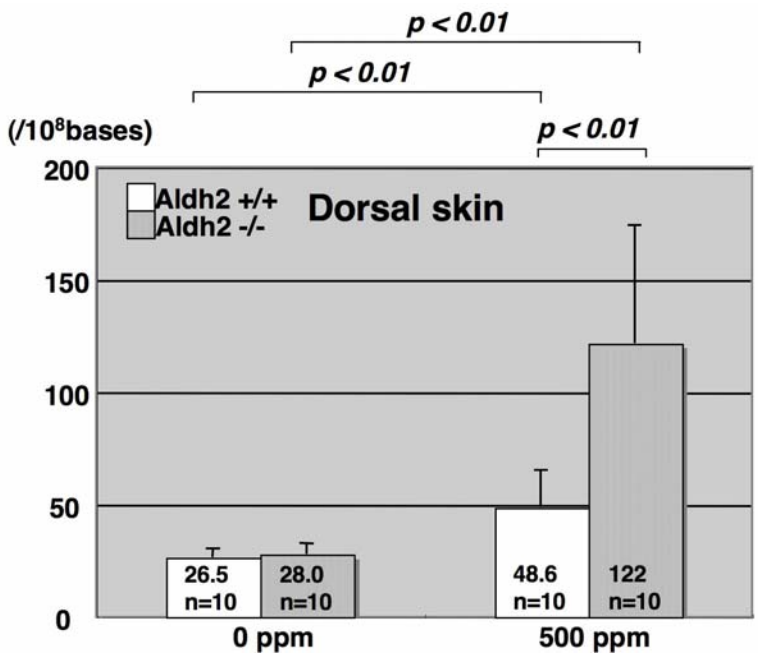

Figure 6. The N^2-ethylidene-dG level in the dorsal skin DNA . The levels of N^2-ethylidene-dG in the dorsal skin chromosomal DNA from both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice were analyzed.

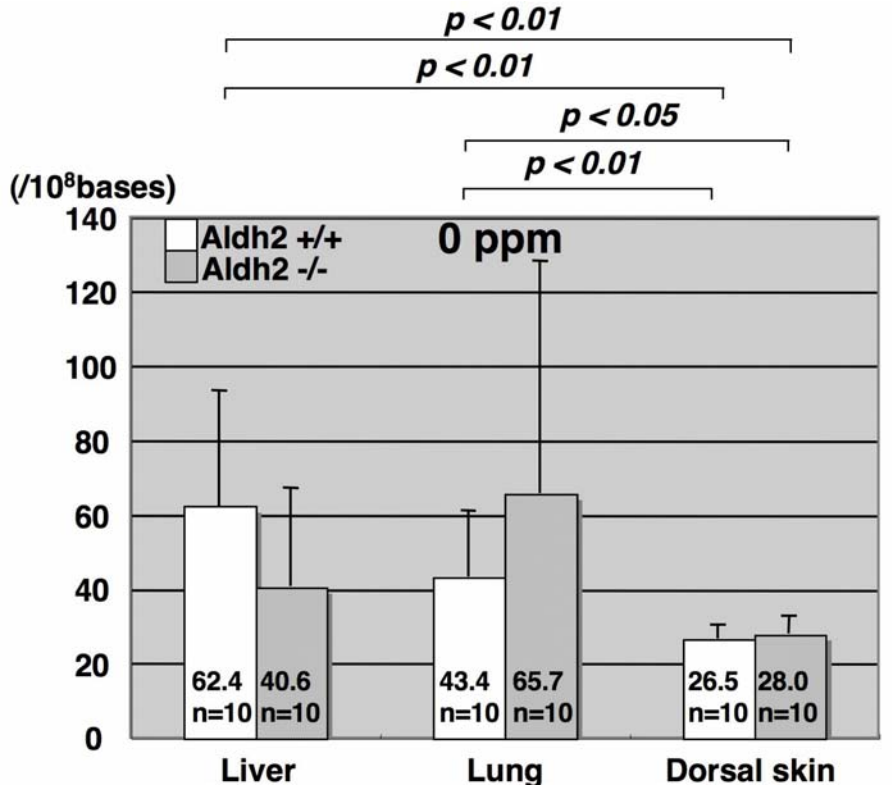

Figure 7. Comparison of the N^2-ethylidene-dG level among tissues from 0 ppm exposure groups. The data from figure 3, 5, and 6 were statistically analyzed as described in "Methods and Materials".

pathway but also by microsomal ethanol oxidizing system (MEOS), such as cytochrome P450 (CYP) 2E1. Following alcohol drinking, the MEOS is induced, causing enhancement of metabolic activity of ethanol in the liver (22, 32). Induced MEOS in the liver, might reduce the hepatocyte acetaldehyde concentration when the blood

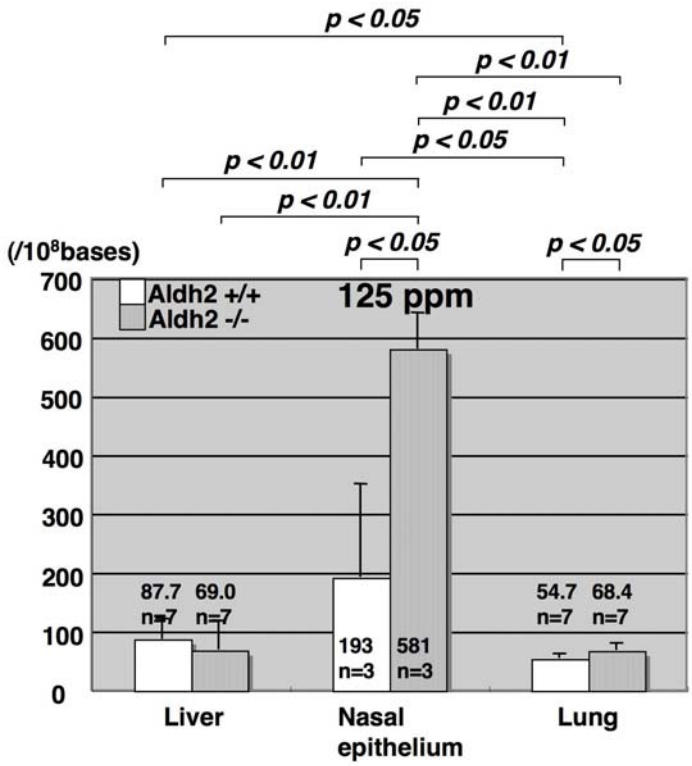

Figure 8. Comparison of the N^2-ethylidene-dG level among tissues from 125 ppm exposure groups. The data from figure 3, 4, and 5 were statistically analyzed as described in "Methods and Materials".

acetaldehyde concentration is low. This might explain the difference in the results of Matsuda *et al.* and our present findings. Because of the requirement of metabolizing aldehyde compounds derived from food, *Aldh2*$^{-/-}$ mice might accumulate induced MEOS in their liver. The induced MEOS may metabolize acetaldehyde in the liver. Therefore, the adduct level can be low in the liver of *Aldh2*$^{-/-}$ when compared to *Aldh2*$^{+/+}$ mice; this was the case in the present study. However, when the mice are fed with alcohol, it is metabolized to acetaldehyde in the liver, suggesting that the liver of alcohol-fed mice might be exposed at a higher levels of acetaldehyde than in the mice exposed to air containing acetaldehyde.

In the nasal epithelium, lung, and dorsal skin, the N^2-ethylidene-dG levels in *Aldh2*$^{-/-}$ mice were higher than those of *Aldh2*$^{+/+}$ mice. The Aldh2 protein is expressed in the liver, lung, heart, kidney, testis, colon, and pancreas, and with less extent in esophagus and stomach (2). Our results suggest that, not only lung but also nasal epithelium and dorsal skin may express the Aldh2b protein. The local metabolism of acetaldehyde in the nasal epithelium, lung and dorsal skin, particularly the accumulation of acetaldehyde caused by *Aldh2* genotype, may be directly associated with elevated N^2-ethylidene-dG levels.

In the liver and lung of both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice, the N^2-ethylidene-dG levels of 0 ppm exposure groups were higher than that in the dorsal skin (Figure 7). The blood volume in the liver and lung is higher than that of dorsal skin. Therefore, the chromosomal DNA may be exposed to acetaldehyde derived from food more frequently in the liver and lung than in the dorsal skin; this resultsin elevated levels of DNA-adduct in the liver and lung compared to dorsal skin even without acetaldehyde exposure. Acetaldehyde easily dissolves in saliva, and the nasal epithelium is the first respiratory organ exposed to acetaldehyde. In the 125 ppm-exposed group, chromosomal DNA of nasal epithelium showed a high level of N^2-ethylidene-dG compared to those from the liver and lung (Figure 8). Similar acetaldehyde inhalation studies in rats also showed that nasal adenocarcinoma occurred in male rats exposed to 750 ppm acetaldehyde for more than 12 months (33-36). Since the lung is the second respiratory organ, chromosomal DNA in the lung is exposed to a high concentration of acetaldehyde in the inhalation experiments. The level of N^2-ethylidene-dG in the lung of both *Aldh2*$^{+/+}$ and *Aldh2*$^{-/-}$ mice exposed to 500 ppm acetaldehyde was higher than that in the liver and the dorsal skin (Figure 9). Our results from acetaldehyde inhalation experiments indicate that the level of DNA adduct production is is in the following order: nasal epithelium > lung > liver and dorsal skin.

The Japan Society for Occupational Health (JSOH) recommends the Occupational Exposure Limits (OELs) as reference values for preventing adverse health effects on workers caused by occupational exposure to chemicals and OEL for acetaldehyde is determined as 50 ppm (37). Threshold Limit Value-Ceiling of American

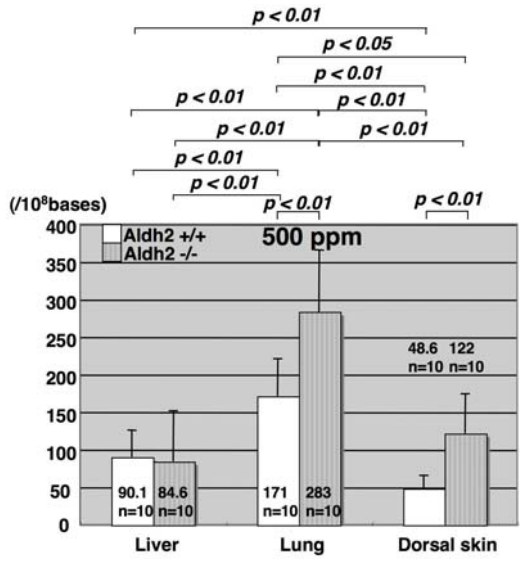

Figure 9. Comparison of the N^2-ethylidene-dG level among tissues from 500 ppm exposure groups. The data from figure 3, 5, and 6 were statistically analyzed as described in "Methods and Materials

Conference of Governmental Industrial Hygienists (ACGIH) for acetaldehyde determined as 25 ppm (http://www.acgih.org/home.htm). On the other hand, the Ministry of Health, Labour and Welfare reported that the acetaldehyde amounts of the mainstream smoke and the secondhand smoke of the cigarette were 408 ± 210 and 1,710 ± 105 were μg per cigarette, respectively. The acetaldehyde concentration of the secondhand smoke of the cigarette was 2.1 – 4.6 mg/L (38). Wong *et al.* showed that the acetaldehyde concentration in the breath was 0.22 ± 0.10 μg/L 30 minutes after drinking ethanol (0.3 g/kg body weight) and the t 1/2 elimination for acetaldehyde was 2.25 hours (39). The air containing acetaldehyde at levels of 125 ppm and 500 ppm seems to be high when it compared with a usual exposure level, such as indoor pollutions although it could happen on the labor site. Therefore, individuals with the *ALDH2*2* allele should be aware of an elevated risk of the incidence of cancer in the respiratory system and skin after acetaldehyde inhalation.

6. ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sport, Science and Technology of Japan (18390187 and 20590620 to T.O.and T.K., 18101003 to T.M.). This study was also funded in part by a Research on the association between risk of upper aero-digestive tract cancer and alcohol-metabolizing enzymes, and its clinical significance (to T.O., T.M. and K.K.), grant number 20-10.

7. REFERENCE

1. Morita, M., T. Oyama, N. Kagawa, S. Nakata, K. Ono, M. Sugaya, H. Uramoto, T. Yoshimatsu, T. Hanagiri, K. Sugio, Y. Kakeji & K. Yasumoto: Expression of aldehyde dehydrogenase 2 in the normal esophageal epithelium and alcohol consumption in patients with esophageal cancer. *Front Biosci*, 10, 2319-24 (2005)

2. Oyama, T., T. Isse, N. Kagawa, T. Kinaga, Y. D. Kim, M. Morita, K. Sugio, H. Weiner, K. Yasumoto & T. Kawamoto: Tissue-distribution of aldehyde dehydrogenase 2 and effects of the ALDH2gene-disruption on the expression of enzymes involved in alcohol metabolism. *Front Biosci*, 10, 951-60 (2005)

3. Yokoyama, A., T. Muramatsu, T. Ohmori, H. Makuuchi, S. Higuchi, S. Matsushita, K. Yoshino, K. Maruyama, M. Nakano & H. Ishii: Multiple primary esophageal and concurrent upper aerodigestive tract cancer and the aldehyde dehydrogenase-2 genotype of Japanese alcoholics. *Cancer*, 77, 1986-90 (1996)

4. Yokoyama, A., T. Muramatsu, T. Ohmori, T. Yokoyama, K. Okuyama, H. Takahashi, Y. Hasegawa, S. Higuchi, K. Maruyama, K. Shirakura & H. Ishii: Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis*, 19, 1383-7 (1998)

5. Yokoyama, A., H. Watanabe, H. Fukuda, T. Haneda, H. Kato, T. Yokoyama, T. Muramatsu, H. Igaki & Y. Tachimori: Multiple cancers associated with esophageal and oropharyngolaryngeal squamous cell carcinoma and the aldehyde dehydrogenase-2 genotype in male Japanese drinkers. *Cancer Epidemiol Biomarkers Prev*, 11, 895-900 (2002)

6. Blum, H. E.: Hepatocellular carcinoma: susceptibility markers. *IARC Sci Publ*, 154, 241-4 (2001)

7. Katoh, T., S. Kaneko, K. Kohshi, M. Munaka, K. Kitagawa, N. Kunugita, K. Ikemura & T. Kawamoto: Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. *Int J Cancer*, 83, 606-9 (1999)

8. Novak, R. F. & K. J. Woodcroft: The alcohol-inducible form of cytochrome P450 (CYP 2E1): role in toxicology and regulation of expression. *Arch Pharm Res*, 23, 267-82 (2000)

9. Agarwal, D. P. & H. W. Goedde: Pharmacogenetics of alcohol metabolism and alcoholism. *Pharmacogenetics*, 2, 48-62 (1992)

10. Yin, S. J.: Alcohol dehydrogenase: enzymology and metabolism. *Alcohol Alcohol Suppl*, 2, 113-9 (1994)

11. Bosron, W. F. & T. K. Li: Catalytic properties of human liver alcohol dehydrogenase isoenzymes. *Enzyme*, 37, 19-28 (1987)

12. Klyosov, A. A., L. G. Rashkovetsky, M. K. Tahir, W. M. Keung, L. G. Rashkovetsky, W. Maret & A. A. Klyosov: Possible role of liver cytosolic and mitochondrial aldehyde dehydrogenases in acetaldehyde metabolism Human liver aldehyde dehydrogenases: new method of

purification of the major mitochondrial and cytosolic enzymes and re-evaluation of their kinetic properties. *Biochemistry*, 35, 4445-56 (1996)

13. Goedde, H. W., D. P. Agarwal, G. Fritze, D. Meier-Tackmann, S. Singh, G. Beckmann, K. Bhatia, L. Z. Chen, B. Fang, R. Lisker & *et al.*: Distribution of ADH2 and ALDH2genotypes in different populations. *Hum Genet*, 88, 344-6 (1992)

14. Crabb, D. W., H. J. Edenberg, W. F. Bosron & T. K. Li: Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive ALDH2(2) allele is dominant. *J Clin Invest*, 83, 314-6 (1989)

15. IARC: International Agency for Research on Cancer. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxidate. In: IARC Monographs on the Evaluation on the Carcinogenic Risks to Humans. IARC, Lyon, France (1999)

16. Brooks, P. J. & J. A. Theruvathu: DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. *Alcohol*, 35, 187-93 (2005)

17. Fang, J. L. & C. E. Vaca: Detection of DNA adducts of acetaldehyde in peripheral white blood cells of alcohol abusers. *Carcinogenesis*, 18, 627-32 (1997)

18. Matsuda, T., M. Kawanishi, T. Yagi, S. Matsui & H. Takebe: Specific tandem GG to TT base substitutions induced by acetaldehyde are due to intra-strand crosslinks between adjacent guanine bases. *Nucleic Acids Res*, 26, 1769-74 (1998)

19. Wang, M., E. J. McIntee, G. Cheng, Y. Shi, P. W. Villalta & S. S. Hecht: Identification of DNA adducts of acetaldehyde. *Chem Res Toxicol*, 13, 1149-57 (2000)

20. Secretan, B., K. Straif, R. Baan, Y. Grosse, F. El Ghissassi, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet & V. Cogliano: A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol*, 10, 1033-4 (2009)

21. Sakamoto, T., M. Hara, Y. Higaki, M. Ichiba, M. Horita, T. Mizuta, Y. Eguchi, T. Yasutake, I. Ozaki, K. Yamamoto, S. Onohara, S. Kawazoe, H. Shigematsu, S. Koizumi & K. Tanaka: Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2on hepatocellular carcinoma in a Japanese population. *Int J Cancer*, 118, 1501-7 (2006)

22. Munaka, M., K. Kohshi, T. Kawamoto, S. Takasawa, N. Nagata, H. Itoh, S. Oda & T. Katoh: Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and the risk of hepatocellular carcinoma. *J Cancer Res Clin Oncol*, 129, 355-60 (2003)

23. Chen, C. H., G. R. Budas, E. N. Churchill, M. H. Disatnik, T. D. Hurley & D. Mochly-Rosen: Activation of aldehyde dehydrogenase-2 reduces ischemic damage to the heart. *Science*, 321, 1493-5 (2008)

24. Matsuda, T., A. Matsumoto, M. Uchida, R. A. Kanaly, K. Misaki, S. Shibutani, T. Kawamoto, K. Kitagawa, K. I. Nakayama, K. Tomokuni & M. Ichiba: Increased formation of hepatic N2-ethylidene-2'-deoxyguanosine DNA adducts in aldehyde dehydrogenase 2-knockout mice treated with ethanol. *Carcinogenesis*, 28, 2363-6 (2007)

25. Matsuda, T., H. Yabushita, R. A. Kanaly, S. Shibutani & A. Yokoyama: Increased DNA damage in ALDH2-deficient alcoholics. *Chem Res Toxicol*, 19, 1374-8 (2006)

26. Wang, M., N. Yu, L. Chen, P. W. Villalta, J. B. Hochalter & S. S. Hecht: Identification of an acetaldehyde adduct in human liver DNA and quantitation as N2-ethyldeoxyguanosine. *Chem Res Toxicol*, 19, 319-24 (2006)

27. Kitagawa, K., T. Kawamoto, N. Kunugita, T. Tsukiyama, K. Okamoto, A. Yoshida, K. Nakayama & K. Nakayama: Aldehyde dehydrogenase (ALDH) 2 associates with oxidation of methoxyacetaldehyde; *in vitro* analysis with liver subcellular fraction derived from human and Aldh2gene targeting mouse. *FEBS Lett*, 476, 306-11 (2000)

28. Isse, T., K. Matsuno, T. Oyama, K. Kitagawa & T. Kawamoto: Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity shows high acetaldehyde level in blood, brain, and liver after ethanol gavages. *Alcohol Clin Exp Res*, 29, 1959-64 (2005)

29. Isse, T., T. Oyama, K. Matsuno, M. Ogawa, R. Narai-Suzuki, T. Yamaguchi, T. Murakami, T. Kinaga, I. Uchiyama & T. Kawamoto: Paired acute inhalation test reveals that acetaldehyde toxicity is higher in aldehyde dehydrogenase 2 knockout mice than in wild-type mice. *J Toxicol Sci*, 30, 329-37 (2005)

30. Isse, T., T. Oyama, K. Matsuno, I. Uchiyama & T. Kawamoto: Aldehyde dehydrogenase 2 activity affects symptoms produced by an intraperitoneal acetaldehyde injection, but not acetaldehyde lethality. *J Toxicol Sci*, 30, 315-28 (2005)

31. Oyama, T., T. Isse, M. Ogawa, M. Muto, I. Uchiyama & T. Kawamoto: Susceptibility to inhalation toxicity of acetaldehyde in Aldh2knockout mice. *Front Biosci*, 12, 1927-34 (2007)

32. Druesne-Pecollo, N., B. Tehard, Y. Mallet, M. Gerber, T. Norat, S. Hercberg & P. Latino-Martel: Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. *Lancet Oncol*, 10, 173-80 (2009)

33. Appelman, L. M., R. A. Woutersen & V. J. Feron: Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. *Toxicology*, 23, 293-307 (1982)

34. Woutersen, R. A., L. M. Appelman, V. J. Feron & C. A. Van der Heijden: Inhalation toxicity of acetaldehyde in rats.

II. Carcinogenicity study: interim results after 15 months. *Toxicology*, 31, 123-33 (1984)

35. Woutersen, R. A., L. M. Appelman, A. Van Garderen-Hoetmer & V. J. Feron: Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology*, 41, 213-31 (1986)

36. Woutersen, R. A. & V. J. Feron: Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. *Toxicology*, 47, 295-305 (1987)

37. Health, J. S. f. O.: Recommendation of occupational exposure limits (2007-2008) *J Occup Health*, 49, 328-44 (2007)

38. Nakanishi, J., N. Shinohara & M. Naya: Acetaldehyde. In: Acetaldehyde. Ed: N. I. o. A. I. S. a. T. a. R. C. f. R. management. Maruzen Co., Ltd, Tokyo, Japan (2007)

39. Wong, M. K., B. K. Scott & C. M. Peterson: Breath acetaldehyde following ethanol consumption. *Alcohol*, 9, 189-92(1992)

Abbreviations: ALDH2, aldehyde dehydrogenase 2; *Aldh2*KO mice, *Aldh2*knockout mice; N^2-ethylidene-dG, N^2-ethylidene-2'-deoxyguanosine; MEOS, microsomal ethanol oxidizing system; CYP, cytochrome P450; OEL, Occupational Exposure Limits.

Key Words: aldehyde dehydrogenase 2, *Aldh2*knockout mice, DNA adduct, N^2-ethylidene-dG

Send correspondence to: Tsunehiro Oyama, MDepartment of Environmental Health, School of Medicine, University of Occupational and Environmental Health, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, 807-8555, Japan, Tel: 93-691-7429, Fax: 93-692-9341, E-mail: oyama@med.uoeh-u.ac.jp