

# Combination of ADH1B\*2/ALDH2\*2 polymorphisms alters acetaldehyde-derived DNA damage in the blood of Japanese alcoholics

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The acetaldehyde associated with alcoholic beverages is an evident carcinogen for the esophagus. Genetic polymorphisms of the alcohol dehydrogenase 1B (*ADH1B*) and aldehyde dehydrogenase 2 (*ALDH2*) genes are associated with the risk of esophageal cancer. However, the exact mechanism via which these genetic polymorphisms affect esophageal carcinogenesis has not been elucidated. *ADH1B\*2* is involved in overproduction of acetaldehyde due to increased ethanol metabolism into acetaldehyde, and *ALDH2\*2* is involved in accumulation of acetaldehyde due to the deficiency of acetaldehyde metabolism. Acetaldehyde can interact with DNA and form DNA adducts, resulting in DNA damage. *N*<sup>2</sup>-ethylidene-2'-deoxyguanosine (*N*<sup>2</sup>-ethylidene-dG) is the most abundant DNA adduct derived from acetaldehyde. Therefore, we quantified *N*<sup>2</sup>-ethylidene-dG levels in blood samples from 66 Japanese alcoholic patients using liquid chromatography/electrospray tandem mass spectrometry, and investigated the relationship between *N*<sup>2</sup>-ethylidene-dG levels and *ADH1B* and *ALDH2* genotypes. The median *N*<sup>2</sup>-ethylidene-dG levels (25th percentile, 75th percentile) in patients with *ADH1B\*1/\*1* plus *ALDH2\*1/\*1*, *ADH1B\*2* carrier plus *ALDH2\*1/\*1*, *ADH1B\*1/\*1* plus *ALDH2\*1/\*2*, and *ADH1B\*2* carrier plus *ALDH2\*1/\*2* were 2.14 (0.97, 2.37)/10<sup>7</sup> bases, 2.38 (1.18, 2.98)/10<sup>7</sup> bases, 5.38 (3.19, 6.52)/10<sup>7</sup> bases, and 21.04 (12.75, 34.80)/10<sup>7</sup> bases, respectively. In the *ALDH2\*1/\*2* group, *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in *ADH1B\*2* carriers than in the *ADH1B\*1/\*1* group ( $P < 0.01$ ). *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the *ALDH2\*1/\*2* group than in the *ALDH2\*1/\*1* group, regardless of *ADH1B* genotype (*ADH1B\*1/\*1*,  $P < 0.05$ ; *ADH1B\*2* carriers,  $P < 0.01$ ) *N*<sup>2</sup>-ethylidene-dG levels in blood DNA of the alcoholics was remarkably higher in individuals with a combination of the *ADH1B\*2* and *ALDH2\*2* alleles. These results provide a new perspective on the carcinogenicity of the acetaldehyde associated with alcoholic beverages, from the aspect of DNA damage. (*Cancer Sci* 2012; 103: 1651–1655)

Esophageal squamous cell carcinoma (SCC) occurs 3.7–9.5 times more frequently in East-Asian countries, such as Japan, China, Taiwan, and Korea, than in Western countries.<sup>(1)</sup> Alcohol consumption is a well-established risk factor for esophageal SCC; moreover, in October 2009, the International Agency for Research on Cancer certified that the acetaldehyde associated with alcoholic beverages is an evident carcinogen for the head and neck region and for the esophagus.<sup>(2)</sup> However, the carcinogenic mechanism of acetaldehyde in these regions remains unclear.

Orally ingested ethanol is metabolized to acetaldehyde mainly by alcohol dehydrogenase 1B (*ADH1B*) and alcohol dehydrogenase 1C (*ADH1C*) in the liver. Subsequently,

acetaldehyde is metabolized to acetate by aldehyde dehydrogenase 2 (*ALDH2*) in the liver. The *ADH1B* and *ALDH2* genes contain single-nucleotide polymorphisms (SNPs) that modulate the enzymatic activity of their protein products. The variant *ADH1B\*2* increases ethanol metabolism to produce acetaldehyde by 40 times because of an amino acid substitution of histidine for arginine at position 47 of the protein. The variant *ALDH2\*2* is deficient in the activity of acetaldehyde catabolism because of an amino acid substitution of lysine for glutamine at position 487. These variants are thought to play protective roles against alcoholism because of the unpleasant flushing response associated with acetaldehydemia after drinking.<sup>(3)</sup>

In East-Asian countries, *ADH1B\*2* and *ALDH2\*2* are prevalent genotypes found in approximately 90% and 50% of these populations, respectively.<sup>(4)</sup> Despite the protection against alcohol intake afforded by these genotypes, continuous alcohol consumption by these individuals leads to increased exposure to carcinogenic acetaldehyde. Therefore, this genetic background is considered a cause of the high incidence of esophageal SCC in East Asia.

Acetaldehyde is a highly reactive electrophile that can interact with DNA to form DNA adducts, such as *N*<sup>2</sup>-ethyl-2'-deoxyguanosine (*N*<sup>2</sup>-Et-dG),<sup>(5,6)</sup>  $\alpha$ -S- and  $\alpha$ -R-methyl- $\gamma$ -hydroxy-1, *N*<sup>2</sup>-propano-2'-deoxyguanosine ( $\alpha$ -S-Me- $\gamma$ -OH-PdG and  $\alpha$ -R-Me- $\gamma$ -OH-PdG),<sup>(7,8)</sup> and *N*<sup>2</sup>-ethylidene-2'-deoxyguanosine (*N*<sup>2</sup>-ethylidene-dG).<sup>(7)</sup> *N*<sup>2</sup>-Et-dG blocks DNA synthesis and induces DNA mutation.<sup>(9–11)</sup>  $\alpha$ -S-Me- $\gamma$ -OH-PdG and  $\alpha$ -R-Me- $\gamma$ -OH-PdG induce DNA-protein or DNA–DNA cross-links and induce DNA mutation.<sup>(12)</sup> We reported previously that the levels of the *N*<sup>2</sup>-Et-dG,  $\alpha$ -S-Me- $\gamma$ -OH-PdG, and  $\alpha$ -R-Me- $\gamma$ -OH-PdG adducts were significantly higher in the leukocytes of *ALDH2*-deficient Japanese alcoholics.<sup>(13)</sup>

Although *N*<sup>2</sup>-ethylidene-dG was the most abundant of the DNA adducts derived from acetaldehyde,<sup>(14)</sup> it was unstable at the nucleoside level and difficult to measure.<sup>(7)</sup> Wang *et al.*<sup>(7)</sup> established a system to quantify *N*<sup>2</sup>-ethylidene-dG by adding NaBH<sub>3</sub>CN, a strong reducing agent that converts *N*<sup>2</sup>-ethylidene-dG to the stable *N*<sup>2</sup>-Et-dG. Since then, the quantification of *N*<sup>2</sup>-ethylidene-dG has become possible.<sup>(14–20)</sup>

We reported previously that *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the liver and stomach of *ALDH2*-deficient mice after oral ethanol ingestion.<sup>(14,20)</sup> In humans, *N*<sup>2</sup>-ethylidene-dG was detected in liver and lung DNA samples<sup>(15,18)</sup> and *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the blood DNA of drinkers<sup>(17)</sup> and significantly lower in the leukocyte DNA of smokers after smoking cessation.<sup>(16)</sup> However, the

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relationship between  $N^2$ -ethylidene-dG levels and SNPs of the *ADH1B* and *ALDH2* genes has not been examined in humans.

$N^2$ -ethylidene-dG is produced from deoxyguanosine and acetaldehyde. Acetaldehyde is ubiquitous in the environment<sup>(21)</sup> and alcoholic beverages and cigarettes are major sources of acetaldehyde for humans. In particular, alcohol consumption may be the most important route of acetaldehyde exposure in humans, as typical alcohol beverages contain over 10 g of ethanol/glass, much of which may be metabolized to acetaldehyde, whereas the level of acetaldehyde in cigarette smoke ranges from 0.6 to 2.1 mg/cigarette.<sup>(22)</sup>  $N^2$ -ethylidene-dG levels in the blood of drinkers were  $5270 \pm 8770$  fmol/ $\mu\text{mol}$  dG,<sup>(17)</sup> which was several times higher than that observed in the smokers who drink only occasionally or not at all ( $1310 \pm 1720$  fmol/ $\mu\text{mol}$  dG).<sup>(16)</sup> Therefore,  $N^2$ -ethylidene-dG could become a good biomarker of acetaldehyde exposure associated with alcohol beverages.

In this study, we quantified  $N^2$ -ethylidene-dG levels in the leukocytes of Japanese alcoholics, as a biomarker of acetaldehyde-derived DNA damage, and investigated the relationship between  $N^2$ -ethylidene-dG levels and SNPs of the *ADH1B* and *ALDH2* genes.

## Materials and Methods

**Patients and *ADH1B* and *ALDH2* genotypes.** The participants in this study were 66 Japanese intoxicated alcoholic men who came to the Kurihama Alcoholism Center of the National Hospital Organization. This study was approved by the Ethics Committee of Kurihama Alcoholism Center for the National Hospital Organization and informed consent was obtained from all participating patients. Information regarding drinking profiles and smoking habits was obtained from the patients, as described previously.<sup>(23)</sup> Blood samples were obtained from patients on the day of admission for alcoholism treatment. *ADH1B* and *ALDH2* genotyping was performed on blood DNA using PCR/RFLP, as described previously.<sup>(24,25)</sup>

**Isolation of DNA.** DNA was isolated from 1 mL of whole blood using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). The procedure was performed according to the manufacturer's instructions, with the exception of the addition of  $\text{NaBH}_3\text{CN}$  to all solutions (final concentration, 100 mM). After the purification step, DNA was dissolved in distilled water.

**DNA adduct standard and its stable isotope.**  $N^2$ -Et-dG and [ $U\text{-}^{15}\text{N}_5$ ]-labeled  $N^2$ -Et-dG were synthesized as described previously.<sup>(6)</sup>

**DNA digestion.** DNA samples (10–15  $\mu\text{g}$ ) were digested to their corresponding 2'-deoxyribonucleoside-3'-monophosphates via the addition of 15  $\mu\text{L}$  of 17 mM sodium succinate and 8 mM  $\text{CaCl}_2$  buffer (pH 6.0) containing micrococcal nuclease (22.5 U) and spleen phosphodiesterase (0.075 U), and [ $U\text{-}^{15}\text{N}_5$ ]-labeled  $N^2$ -Et-dG. Solutions were mixed and incubated for 3 h at 37°C, followed by the addition of alkaline phosphatase (1 U), 10  $\mu\text{L}$  of 0.5 M Tris-HCl (pH 8.5), 5  $\mu\text{L}$  of 20 mM  $\text{ZnSO}_4$ , and 67  $\mu\text{L}$  of distilled water and incubation of solutions for an additional 3 h at 37°C. The mixture was evaporated to 10–20  $\mu\text{L}$  and 100  $\mu\text{L}$  of methanol was added to precipitate proteins. After centrifugation, the methanol fraction (supernatant) was transferred to a new Eppendorf tube. The precipitate was re-extracted using 100  $\mu\text{L}$  of methanol and the methanol fraction was collected into the Eppendorf tube. This methanol solution was evaporated to dryness, resuspended in 50  $\mu\text{L}$  of distilled water, and subjected to liquid chromatography/electrospray tandem mass spectrometry (LC/MS/MS).

**Instrumentation.** Liquid chromatography/electrospray tandem mass spectrometry 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, to 80% methanol from 30 to 35 min, and finally returned to the original starting conditions (5:95) for the remaining 11 min. The total run time was 46 min. Sample injection volumes of 20  $\mu\text{L}$  each were separated on a Shim-pack XR-ODS column ( $3.0 \times 75$  mm, 2.2  $\mu\text{m}$ ; Shimadzu) and eluted at a flow rate of 0.2 mL/min. Mass spectral analyses were performed in a positive ion mode using nitrogen as the nebulizing gas. The temperature of the ion source was 130°C, the temperature of the desolvation gas was 380°C, and the cone voltage was operated at a constant 35 V. Nitrogen was also used as the desolvation gas (700 L/h) and cone gas (35 L/h), and argon was used as the collision gas, at a collision cell pressure of  $1.5 \times 10^{-3}$  mbar. Positive ions were acquired in multiple-reaction monitoring mode. The multiple-reaction monitoring transitions monitored were as follows: [ $^{15}\text{N}_5$ ]- $N^2$ -Et-dG,  $m/z$  301  $\rightarrow$  185 and  $N^2$ -Et-dG,  $m/z$  296  $\rightarrow$  180. The amount of  $N^2$ -Et-dG was quantified using the ratio of the peak area of  $N^2$ -Et-dG to that of [ $U\text{-}^{15}\text{N}_5$ ]-labeled  $N^2$ -Et-dG. The QuanLynx software (version 4.0; Waters/Micromass) was used to create a standard curve and to calculate the concentration of  $N^2$ -Et-dG. The amount of deoxyguanosine was monitored using a Shimadzu SPD-10A UV-Visible detector that was set in place before the tandem MS.

**Statistical analyses.** Statistical analyses were performed using SPSS statistics software (version 17; SPSS Inc., Chicago, IL, USA). Values were expressed as means and standard deviations or medians plus 25th and 75th percentiles (Q1, Q3). Analysis of variance (ANOVA) and the Kruskal-Wallis test were used to compare normally distributed data and non-normally distributed data, respectively, among the groups with different *ADH1B* and *ALDH2* genotype combinations; in addition, *post hoc* paired comparisons were performed using Tukey's method for ANOVA and Holm's method for the Kruskal-Wallis test.<sup>(23)</sup> Because  $N^2$ -ethylidene-dG levels were not normally distributed, the Mann-Whitney test was used to compare  $N^2$ -ethylidene-dG levels among genotype groups. Holm's method was used to adjust the *P*-values for multiplicity.<sup>(23)</sup> An adjusted *P*-value < 0.05 was considered significant.

## Results

**Clinical characteristics.** Table 1 lists the patients' characteristics. There were 50 patients in the *ALDH2*\*1/\*1 group, 16 patients in the *ALDH2*\*1/\*2 group, and no patients in the *ALDH2*\*2/\*2 group. Among the 50 patients with the *ALDH2*\*1/\*1 genotype, 13 patients were homozygous for *ADH1B*\*1 (*ADH1B*\*1/\*1 + *ALDH2*\*1/\*1; Group 1) and 37 patients were *ADH1B*\*2 carriers (*ADH1B*\*1/\*2, 14; *ADH1B*\*2/\*2, 23) (*ADH1B*\*2 carriers + *ALDH2*\*1/\*1; Group 2). Among the 16 patients with the *ALDH2*\*1/\*2 genotype, eight patients were homozygous for *ADH1B*\*1 (*ADH1B*\*1/\*1 + *ALDH2*\*1/\*2; Group 3) and eight patients were *ADH1B*\*2 carriers (*ADH1B*\*1/\*2, 4; *ADH1B*\*2/\*2, 4) (*ADH1B*\*2 carriers + *ALDH2*\*1/\*2; Group 4).

In the *ALDH2*\*1/\*1 group, the mean body weight was significantly lower in Group 2 than in Group 1 ( $P < 0.05$ ), whereas no such difference was observed in the *ALDH2*\*1/\*2 group. There were no significant differences among the four groups regarding age, height, amount of alcohol consumed in the previous 24 h, interval from the last drink, and number of cigarettes smoked during the previous 24 h.

**Level of the  $N^2$ -ethylidene-dG adduct and combinations of *ADH1B* and *ALDH2* genotypes.**  $N^2$ -ethylidene-dG was converted to  $N^2$ -Et-dG by adding  $\text{NaBH}_3\text{CN}$  during the DNA isolation step and the amount of  $N^2$ -Et-dG was measured. Because the amount of  $N^2$ -Et-dG is, by far, lower than that of

**Table 1. Relationship between clinical characteristics and genotypes of *ADH1B* and *ALDH2* (n = 66)**

	Group 1 (n = 13)	Group 2 (n = 37)	Group 3 (n = 8)	Group 4 (n = 8)	P-value
<i>ADH1B</i>	*1/*1	*1/*2 + *2/*2	*1/*1	*1/*2 + *2/*2	–
<i>ALDH2</i>	*1/*1	*1/*1	*1/*2	*1/*2	–
Age (years)	50.0 (40.0, 57.0)	56.0 (43.0, 63.0)	55.0 (44.0, 57.0)	54.0 (50.5, 63.5)	0.557
Height (cm)	167.9 ± 5.6	166.0 ± 6.2	168.8 ± 7.0	169.1 ± 5.0	0.409
Weight (kg)	66.9 ± 16.2†	57.3 ± 7.7†	58.8 ± 10.1	59.8 ± 9.3	0.050
Amount of ethanol consumed in the previous 24 h (mL)	120 (110, 180)	100 (80, 160)	120 (105, 140)	150 (95, 205)	0.364
Interval from the last drink (h)	12.0 (8.0, 14.0)	13.0 (10.0, 24.0)	9.5 (8.0, 14.0)	10.5 (7.5, 14.0)	0.285
Number of cigarettes smoked in the previous 24 h (pieces)	20.8 ± 10.2	15.6 ± 13.3	20.6 ± 12.1	20.6 ± 10.2	0.430

†P < 0.05 for pairwise comparisons adjusted for multiplicity. Values are means ± standard deviation (SD) or medians (25th percentile, 75th percentile); P-values are for homogeneity among the four groups based on analysis of variance (ANOVA) and the Kruskal–Wallis test, respectively. *ADH1B*, alcohol dehydrogenase 1B; *ALDH2*, aldehyde dehydrogenase 2.

*N*<sup>2</sup>-ethylidene-dG in the human body,<sup>(15)</sup> the measured *N*<sup>2</sup>-Et-dG level was considered as a surrogate marker of the *N*<sup>2</sup>-ethylidene-dG adduct (Fig. 1).

The median *N*<sup>2</sup>-ethylidene-dG levels (Q1, Q3) were 2.14 (0.97, 2.37)/10<sup>7</sup> bases in Group 1, 2.38 (1.18, 2.98)/10<sup>7</sup> bases in Group 2, 5.38 (3.19, 6.52)/10<sup>7</sup> bases in Group 3, and 21.04 (12.75, 34.80)/10<sup>7</sup> bases in Group 4 (Fig. 2).

There was no significant difference in *N*<sup>2</sup>-ethylidene-dG levels between Group 1 and Group 2. On the other hand, *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in Group 4 than in Group 3 (*P* < 0.01).

*N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the *ALDH2*\*1/\*2 group than in the *ALDH2*\*1/\*1 group, regardless of *ADH1B* genotype (*ADH1B*\*1/\*1, *P* < 0.05; *ADH1B*\*2 carrier, *P* < 0.01).

## Discussion

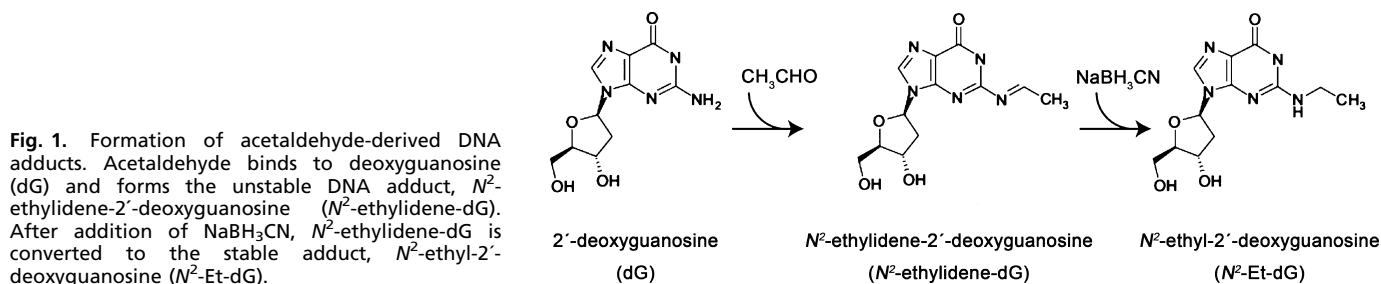
This study demonstrated for the first time the relationship between the levels of *N*<sup>2</sup>-ethylidene-dG and *ADH1B* and *ALDH2* genotypes in human samples. We found that the level of *N*<sup>2</sup>-ethylidene-dG in the DNA of leukocytes from alcoholics was remarkably elevated in individuals with a combination of *ADH1B*\*2 and *ALDH2*\*2 alleles.

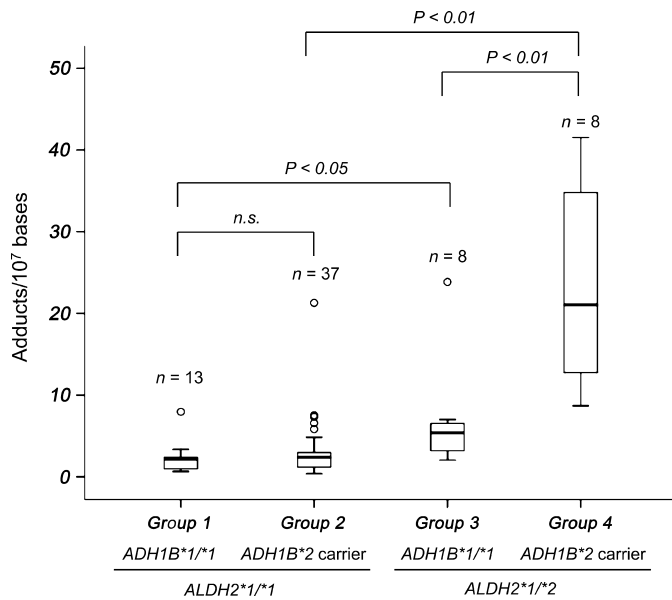
It was reported that *N*<sup>2</sup>-ethylidene-dG was unstable.<sup>(7)</sup> In our *in vitro* experiments in which HL-60 cells (human promyelocytic leukemia cells) were cultured with acetaldehyde contained in medium, the half-life of this adduct was approximately 35 h (Kimiko Hori, Shin'ichiro Miyamoto, Yoshiyuki Yukawa, Manabu Muto, Tsutomu Chiba and Tomonari Matsuda, unpublished data, 2012). However, the levels of *N*<sup>2</sup>-ethylidene-dG in the leukocytes of alcoholics, as assessed in this study, were more abundant than those of *N*<sup>2</sup>-Et-dG (*ALDH2*\*1/\*1, 0.04 ± 0.03/10<sup>7</sup> bases; *ALDH2*\*1/\*2, 0.28 ± 0.11/10<sup>7</sup> bases), *α*-*S*-Me-*γ*-OH-PdG (*ALDH2*\*1/\*1, 0.09 ±

0.01/10<sup>7</sup> bases; *ALDH2*\*1/\*2, 0.20 ± 0.03/10<sup>7</sup> bases), and *α*-*R*-Me-*γ*-OH-PdG (*ALDH2*\*1/\*1, 0.13 ± 0.01/10<sup>7</sup> bases; *ALDH2*\*1/\*2, 0.25 ± 0.03/10<sup>7</sup> bases) in leukocytes from similar populations reported previously.<sup>(13)</sup> Therefore, *N*<sup>2</sup>-ethylidene-dG should be a sensitive biomarker of acetaldehyde exposure. Although the mutagenicity of this adduct remains undetermined, the fact that *N*<sup>2</sup>-ethylidene-dG is converted to *N*<sup>2</sup>-Et-dG, *α*-*S*-Me-*γ*-OH-PdG and *α*-*R*-Me-*γ*-OH-PdG, which are more stable and mutagenic<sup>(7–12,26)</sup> also shows the biological significance of *N*<sup>2</sup>-ethylidene-dG.

Yokoyama *et al.*<sup>(23)</sup> reported an absence of differences in blood acetaldehyde concentration between *ALDH2*\*1/\*2 intoxicated alcoholics carrying the *ADH1B*\*1/\*1 and *ADH1B*\*2 alleles, whereas the slope of the increase in blood acetaldehyde concentration according to increasing concentration of blood ethanol levels was significantly steeper in *ADH1B*\*2 carriers with *ALDH2*\*1/\*2 than in other combinations of *ADH1B* and *ALDH2* genotypes. Other investigators also reported that the combination of *ADH1B*\*2 and *ALDH2*\*1/\*2 evoked the most intense alcohol flushing response, which was associated with an acetaldehydemia reaction.<sup>(3,27)</sup> Therefore, the present data could be interpreted as indicating that the exposure to increased levels of acetaldehyde increases the amount of acetaldehyde-derived DNA adducts, especially in individuals with a combination of the *ADH1B*\*2 and *ALDH2*\*2 alleles.

In the present study, we measured DNA adducts quantitatively in the leukocytes of Japanese alcoholics and showed that the levels of *N*<sup>2</sup>-ethylidene-dG in individuals with *ALDH2*\*1/\*2 were higher than those detected in individuals with *ALDH2*\*1/\*1. Furthermore, individuals with a combined genotype of *ADH1B*\*2 and *ALDH2*\*1/\*2 exhibited the highest levels of *N*<sup>2</sup>-ethylidene-dG. Recently, Weng *et al.* reported that DNA damage (as assessed using an alkaline comet assay) was significantly increased in elderly habitual drinkers with the *ALDH2*\*2 allele. Furthermore, habitual drinkers with a





**Fig. 2.** *N*<sup>2</sup>-ethylidene-2'-deoxyguanosine (*N*<sup>2</sup>-ethylidene-dG) levels according to combinations of the *ADH1B* and *ALDH2* genotypes. *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the blood DNA of alcoholics with the combination of *ADH1B*\*2 and *ALDH2*\*1/\*2 compared with alcoholics with the combination of *ADH1B*\*1/\*1 and *ALDH2*\*1/\*2 ( $P < 0.01$ ). *N*<sup>2</sup>-ethylidene-dG levels were significantly higher in the blood DNA of alcoholics with *ALDH2*\*1/\*2 compared with those with *ALDH2*\*1/\*1, regardless of *ADH1B* genotype (*ADH1B*\*1/\*1,  $P < 0.05$ ; *ADH1B*\*2 carriers,  $P < 0.01$ ). Boxes represent 25th to 75th percentile values (interquartile range [IQR]). The band in the box represents the median. Outer lines represent the highest datum below 1.5 IQR above the 75th percentile and the lowest datum above 1.5 IQR below the 25th percentile. Small circles represent outliers. The Mann-Whitney test was used to compare *N*<sup>2</sup>-ethylidene-dG levels among genotype groups. Holm's method was used to adjust the *P*-values for multiplicity.

combined genotype of *ADH1B*\*2/\*2 and *ALDH2*\*1/\*2 displayed significantly higher levels of DNA damage.<sup>(28)</sup> These results are concordant with the present data. Thus, these results indicate that acetaldehyde-induced DNA damage is closely associated with the combination of *ADH1B*\*2 and *ALDH2*\*1/\*2.

If acetaldehyde-derived DNA adducts are associated with esophageal carcinogenesis, the risk of disease would be highest among the subjects carrying *ADH1B*\*2 plus *ALDH2*\*1/\*2. However, a recent meta-analysis showed that *ADH1B*\*1/\*1 plus *ALDH2*\*1/\*2 was associated with the highest risk of esophageal cancer among heavy drinkers (odds ratio, 12.45;

95% CI, 2.9–53.46) compared with carriers of *ADH1B*\*2 plus *ALDH2*\*1/\*1.<sup>(29)</sup> In individuals with *ALDH2*\*1/\*2, this risk was higher in *ADH1B*\*1/\*1 compared with *ADH1B*\*2 carriers. These results suggest that *N*<sup>2</sup>-ethylidene-dG adduct levels in leukocytes may not be an adequate biomarker of the risk of esophageal cancer. In addition to the acetaldehyde that circulates systemically, which is produced in the liver from orally ingested ethanol, acetaldehyde produced locally by *ADH7*<sup>(30,31)</sup> and *CYP2E1*<sup>(32)</sup> in the mucosa of the upper aerodigestive tract and by ADHs of oral microbes<sup>(33–35)</sup> has been considered to play an important role in the carcinogenicity of the upper aerodigestive tract. Thus, the relationship between locally produced acetaldehyde-derived DNA damage and the risk of esophageal cancer warrants investigation.

Regarding *ADH1B*, the *ADH1B*\*1 allele increases the risk of cancer in the esophagus<sup>(29,36)</sup> and in the head and neck region;<sup>(36)</sup> conversely, Kanda *et al.* reported that *ADH1B*\*2/\*2 increases the risk of pancreatic cancer in heavy drinkers and that the combination of the *ADH1B*\*2/\*2 and *ALDH2*\*2 alleles is associated with the highest carcinogenic risk.<sup>(37)</sup> Therefore, the carcinogenic risk associated with the *ADH1B* genotype may exhibit opposite trends in different organs. In contrast, the relationship between the *ALDH2*\*2 allele and increased carcinogenic risk has been reported in a variety of organs (e.g., esophagus,<sup>(29,36)</sup> head and neck region,<sup>(36)</sup> stomach, lung,<sup>(38)</sup> and pancreas<sup>(39)</sup>), whereas there are no reports of the association of *ALDH2*\*1/\*1 with elevated carcinogenic risk. Thus, the carcinogenic effect of *ALDH2*\*1/\*2 is apparent. In this study, *N*<sup>2</sup>-ethylidene-dG levels in individuals with *ALDH2*\*1/\*2 were significantly higher than those detected in individuals with *ALDH2*\*1/\*1, regardless of *ADH1B* genotype.

In conclusion, the combination of *ADH1B*\*2 and *ALDH2*\*1/\*2 strongly enhanced DNA damage in the leukocytes of alcoholics. Although further investigations are needed to clarify the involvement of exposure to acetaldehyde in the mechanism of esophageal carcinogenesis associated with alcohol drinking, the results of this study are expected to provide a new perspective on the carcinogenicity of the acetaldehyde associated with alcoholic beverages, from the aspect of DNA damage.

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## Disclosure statement

The authors have no conflict of interest.

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