# Low $K_m$ Aldehyde Dehydrogenase (ALDH2) Polymorphism, Alcohol-drinking Behavior, and Chromosome Alterations in Peripheral Lymphocytes


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Excessive drinking of alcohol is now widely known to be one of the major lifestyle choices that can affect health. Among the various effects of alcohol drinking, cytogenetic and other genotoxic effects are of major concern from the viewpoint of prevention of alcohol-related diseases. Alcohol is first metabolized to acetaldehyde, which directly causes various types of chromosomal DNA lesions and alcohol-related diseases, and is then further detoxified to the much less toxic metabolite acetate. About 50% of Oriental people are deficient in the aldehyde-dehydrogenase 2 isozyme (ALDH2) that can most efficiently detoxify acetaldehyde. We have performed a series of experiments to investigate how the genetic deficiency in ALDH2 affects the behavioral pattern for alcohol drinking and the sensitivity of peripheral lymphocytes to the induction of chromosome alterations by exposure to alcohol and alcohol-related chemicals. We found great effects of the ALDH2 genotypes on alcohol sensitivity and alcohol-drinking behavior. We also show that lymphocytes from habitual drinkers with the deficient ALDH2 enzyme had significantly higher frequencies of sister chromatid exchanges than those from ALDH2-proficient individuals. — Environ Health Perspect 104(Suppl 3):563–567 (1996)



Key words: lifestyles, aldehyde dehydrogenase, genetic polymorphism, alcohol drinking, chromosome alterations, sister chromatid exchange, hydroquinone


## Introduction

Drinking of alcohol is one of the major practices that can cause negative health effects (*1–4*). Among the various effects of alcohol drinking are the cytogenetic and other genotoxic effects, which are of major concern from the viewpoint of prevention of alcohol-related diseases (*5–10*). Alcohol is metabolized to acetaldehyde, which directly causes various types of chromosomal DNA lesions and alcohol-related diseases; it is further detoxified to the less toxic metabolite acetate (*11,12*). Recent studies revealed the existence of genetic deficiency in some enzyme species that can convert acetaldehyde to acetate in the human body (*13,14*). About 50% of Oriental people are deficient in the aldehyde-dehydrogenase 2 isozyme (ALDH2) that can most efficiently detoxify acetaldehyde (*12,14,15*). It is therefore possible that cells in individuals who are genetically deficient in such an aldehyde dehydrogenase activity might show an elevated level of chromosome alterations if they drink too heavily.

We have performed a series of experiments to investigate how the genetic deficiency in ALDH2 affects the behavioral pattern for alcohol drinking and the sensitivity of peripheral lymphocytes to the induction of chromosome alterations by exposure to alcohol and alcohol-related chemicals. We show here that significantly smaller proportions of individuals who are deficient in ALDH2 are habitual alcohol drinkers than those who are not deficient and that lymphocytes from individuals with the ALDH2 enzyme who are habitual alcohol drinkers have significantly higher frequencies of sister chromatid exchanges (SCE) than those from ALDH2-proficient individuals who drink alcohol everyday.

## Methods

Blood samples (2–4 ml) were obtained with informed consent from 424 male and 100 female workers (38.8 ± 11.4 and 33.9 ± 13.3 years of age [mean ± SD], respectively) out of 649 workers in a metal plant in Japan. All subjects were not alcoholic at the time of the investigation. DNA was extracted from 100 µl of white blood cell-rich plasma using an Isoquick kit (MicroProbe, Garden Grove, CA). Exon 12 of the ALDH2 gene was amplified by 30 to 35 cycles of polymerase chain reaction (PCR; 1 min at 94°C, 10 sec at 52°C, and 30 sec at 72°C) in a Perkin-Elmer Cetus (Norwalk, CT) Thermal Cycler. Amplification primers were as previously reported (*16*), except that one primer (5′-CCACACTCACAGTTTTCTCTT) contained the substitution of an adenine by a thymine at the underlined portion in order to create a Ksp632I recognition site (5′-CTCTTC) in the typical allele. PCR products were ethanol precipitated and redissolved in distilled water. The reaction mixture containing PCR products, 2 to 3 units of Ksp632I (Boehringer Mannheim, Mannheim, Germany), and the reaction buffer was incubated at 37°C for 3 to 6 hr and then ethanol precipitated. Resuspended samples were separated on gels containing 3% NuSieve GTG agarose (FMC Bioproducts, Rockland, ME) and 1% regular agarose (Sigma, St. Louis, MO), stained with ethidium bromide, and photographed on Polaroid Type 667 film.

For DNA hybridization, the PCR procedure was essentially identical to the procedure described above, except that the annealing temperature was 50°C and there were 40 amplification cycles. Amplification primers were the same as described previously (*16*). PCR products (5–10 µl) were treated with alkali and transferred to nylon membranes, which were then baked and hybridized overnight with the $^{32}$P-ATP labeled probe (Amersham, Bucks, U.K.),


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Abbreviations used: ALDH, aldehyde dehydrogenase; SCE, sister chromatid exchange; PCR, polymerase chain reaction; BrdUrd, 5-bromo-2′-deoxyuridine; PHA, phytohemagglutinin; TAST, Tokyo-University ALDH2-Phenotype Screening Test.

for either ALDH2[1] (5′-GTTTTCACTTC AGTGTATG) or ALDH2[2] allele (5′-GTTTTCACTTTAGTGTATG). The membranes were washed three times at room temperature, washed with 1×SSC (standard saline citrate) containing 0.1% sodium dodecyl sulfate (SDS) for 30 min at 48°C, and exposed to X-ray film overnight.

A rapid and simple screening method for detecting an ALDH2 deficiency is necessary for epidemiological studies in which a large number of subjects will be investigated. The patch test has been used widely for this purpose (*17*). Also, we have developed a simple self-administered questionnaire consisting of 13 short questions (*18*). We used these two screening methods for detecting the ALDH2 deficiency in this study.

Heparinized peripheral blood samples were obtained from each blood donor. Whole blood cultures were set up according to our standard experimental protocol (*19–21*). Briefly, whole blood (0.2–0.3 ml; final lymphocyte concentration $5\times10^5$/ml) was added to 5 ml of RPMI 1640 tissue culture medium containing 15% fetal bovine serum and antibiotics. When necessary for differential staining of sister chromatids, the culture medium also contained 20 μM 5-bromo-2′-deoxyuridine (BrdUrd). Proliferation was initiated by adding 3% phytohemagglutinin (PHA-M). Colcemid ($2\times10^{-7}$ M) was added usually at the 24th hr and cells were fixed at the 52nd hr after the stain of cultures. In preliminary and concomitant experiments using sister chromatid differential staining of BrdUrd-incorporated chromosomes (*19*), we confirmed that these 52 hr cultures contained exclusively first division metaphases. For examining SCEs, cells were cultured in BrdUrd-containing medium for 72 hr and colcemid was added for the last 3 hr.

## Results and Discussion

PCR amplification using a mutated primer yielded sufficient products, of which 135 basepairs (bp) were cut into 112 and 23 bp fragments by the restriction enzyme Ksp632I, only in the typical allele (ALDH2[1]) (*22,23*). The three ALDH2 genotypes were distinguishable and the results agreed well with those obtained by DNA hybridization. Further studies of 92 subjects revealed complete consistency, including 38 of the typical homozygotes (ALDH2[1]/ALDH2[1]), 38 of the heterozygotes (ALDH2[1]/ALDH2[2]), and 16 of the atypical homozygotes (ALDH2[2]/ALDH2[2]).

Gene frequencies of the typical and atypical allele calculated from the genotype frequencies were 0.743 and 0.267, respectively, for males and 0.790 and 0.210, respectively, for females. Deviation from Hardy-Weinberg's prediction was not statistically significant in either sex ($\chi^2 = 0.062$ and 0.126; $df = 1$, $p > 0.5$).

In males, the frequency of alcohol-associated symptoms such as facial flushing generally increased in the order of typical homozygote, heterozygote, and then atypical homozygote. In females, the differences between the typical homozygote and the heterozygote was similarly significant among most of the symptoms.

Drinking frequency was strongly affected by the ALDH2 genotype, especially in the males. The frequency of those who drank 6 to 7 days/week was significantly lower in the heterozygote than in the typical homozygote and significantly lower in the atypical homozygote than in the heterozygote. The mean amounts of alcohol consumption also increased significantly in the order of atypical homozygote, the heterozygote, the typical homozygote in both sexes; however, the differences among the genotypes were more evident in males than in females.

In our subjects who had kindly given informed consent for cytogenetic investigation, ALDH2 deficiency testing, and lifestyle-information gathering, about 50% [52 and 53% by the patch test and TAST (Tokyo-University ALDH2-Phenotype Screening Test) (*18*), respectively] were habitual alcohol drinkers who drank 40 to 50 g of net alcohol per day. Half of the subjects were found to be deficient in ALDH2 determined by the patch test (50%) or TAST screening (52%). We also observed a significant difference in the proportion of ALDH2-deficient persons between habitual and nonhabitual alcohol drinkers (Table 1). Twenty-six percent of the habitual drinkers were ALDH2 deficient compared to 72% of nonhabitual drinkers. It is noteworthy to realize that about 28% of ALDH2-deficient people who did not drink very much alcohol were screened as habitual drinkers.

**Table 1.** Relationship between ALDH2 and habits of alcohol drinking in individuals.

| | ALDH2 | |
|---|---|---|
| Alcohol consumption | Number (+)[a] (age)[c] | Number (–)[b] (age) |
| Almost every day | 43 (44.26 ± 7.00) | 15 (47.73 ± 8.00) |
| Several times per week or never | 15 (43.67 ± 5.67) | 38 (40.66 ± 8.82) |

Statistical significance determined by $\chi^2$-test ($p < 0.001$). [a]ALDH2[1]/ALDH2[1]. [b]ALDH2[1]/ALDH2[2] or ALDH2[2]/ALDH2[2]. [c]Mean ± SD.

When baseline and hydroquinone-induced frequencies of SCE in peripheral lymphocytes were plotted as a function of daily consumption of alcohol, the increasing tendency of SCE was more marked for ALDH2-deficient persons than for nondeficient ones (Tables 2, 3, Figure 1). This difference was also observed with the data screened by the patch and TAST tests.

We have already found habitual cigarette smoking to have significant effects on the induction of SCE in peripheral lymphocytes. We thus analyzed the effect of alcohol drinking and ALDH2 deficiency in lymphocytes separately from smokers and nonsmokers. The SCE data from smokers was corrected on the assumption that daily smoking of 20 cigarettes/day caused an increase of 1 SCE/cell, according to our previous finding on the SCE frequency in lymphocytes from smokers (*24*). It was generally confirmed that the effect of alcohol drinking on the SCE frequencies was more proficient in ALDH2-deficient lymphocytes than in nondeficient ones, even after controlling the effect of cigarette smoking (Tables 4, 5). This general tendency was similarly found in sample subjects screened by the patch test or by the TAST test.

In conclusion, we found great effects of the ALDH2 genotype on alcohol sensitivity and alcohol-drinking behavior. We also found significantly higher frequencies of SCE in the lymphocytes from the ALDH2-deficient habitual drinkers than in those from the ALDH2-proficient individuals who drank every day. Further extensive studies are required to clarify whether the ALDH2 genotype affects the development of alcohol-related health problems such as cancer or cardiovascular diseases.

**Table 2.** Effects of alcohol consumption on the induction of sister chromatid exchanges in lymphocytes based on ALDH2 status.

| Alcohol consumption | Patch test | | | TAST | | | Genotype | | |
|---|---|---|---|---|---|---|---|---|---|
| | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | |
| Almost every day | 10.29 ± 1.61 (36)[a] | 11.44 ± 1.99 (21) | $p=0.020$[b]* | 10.18 ± 1.37 (41) | 11.44 ± 1.98 (26) | $p=0.007$* | 10.17 ± 1.36 (43) | 12.34 ± 2.03 (15) | $p=0.003$* |
| Several times per week or never | 10.14 ± 1.41 (18) | 9.92 ± 1.55 (34) | $p=0.630$ | 10.08 ± 1.83 (18) | 10.04 ± 1.45 (41) | $p=0.913$ | 9.96 ± 1.86 (15) | 10.11±1.46 (38) | $p=0.759$ |
| | $p=0.739$ | $p=0.003$* | | $p=0.820$ | $p=0.001$* | | $p=0.635$ | $p=0.001$* | |

[a]Values in parentheses = *n*. [b]Determined by *t*-test. *Denotes statistically significant values.

**Table 3.** Effects of alcohol consumption on the induction of sister chromatid exchanges in hydroquinone-treated lymphocytes based on ALDH2 status.[a]

| Alcohol consumption | Patch test | | | TAST | | | Genotype | | |
|---|---|---|---|---|---|---|---|---|---|
| | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | |
| Almost every day | 13.40 ± 1.79 (30)[b] | 14.98 ± 2.68 (15) | $p=0.023$[c]* | 13.14 ± 1.33 (32) | 14.94 ± 2.76 (21) | $p=0.010$* | 13.11 ± 1.44 (36) | 16.50 ± 2.70 (10) | $p=0.003$* |
| Several times per week or never | 12.59 ± 1.25 (12) | 13.18 ± 1.66 (24) | $p=0.281$ | 12.68 ± 1.44 (15) | 13.25 ± 1.57 (28) | $p=0.254$ | 12.39 ± 1.27 (14) | 13.36 ± 1.57 (23) | $p=0.059$ |
| | $p=0.158$ | $p=0.029$* | | $p=0.286$ | $p=0.017$* | | $p=0.111$ | $p=0.005$* | |

[a]Hydroquinone treatment was $5 \times 10^{-5}$ M for 72 hr. [b]Values in parentheses = *n*. [c]Determined by *t*-test. *Denotes statistically significant values.

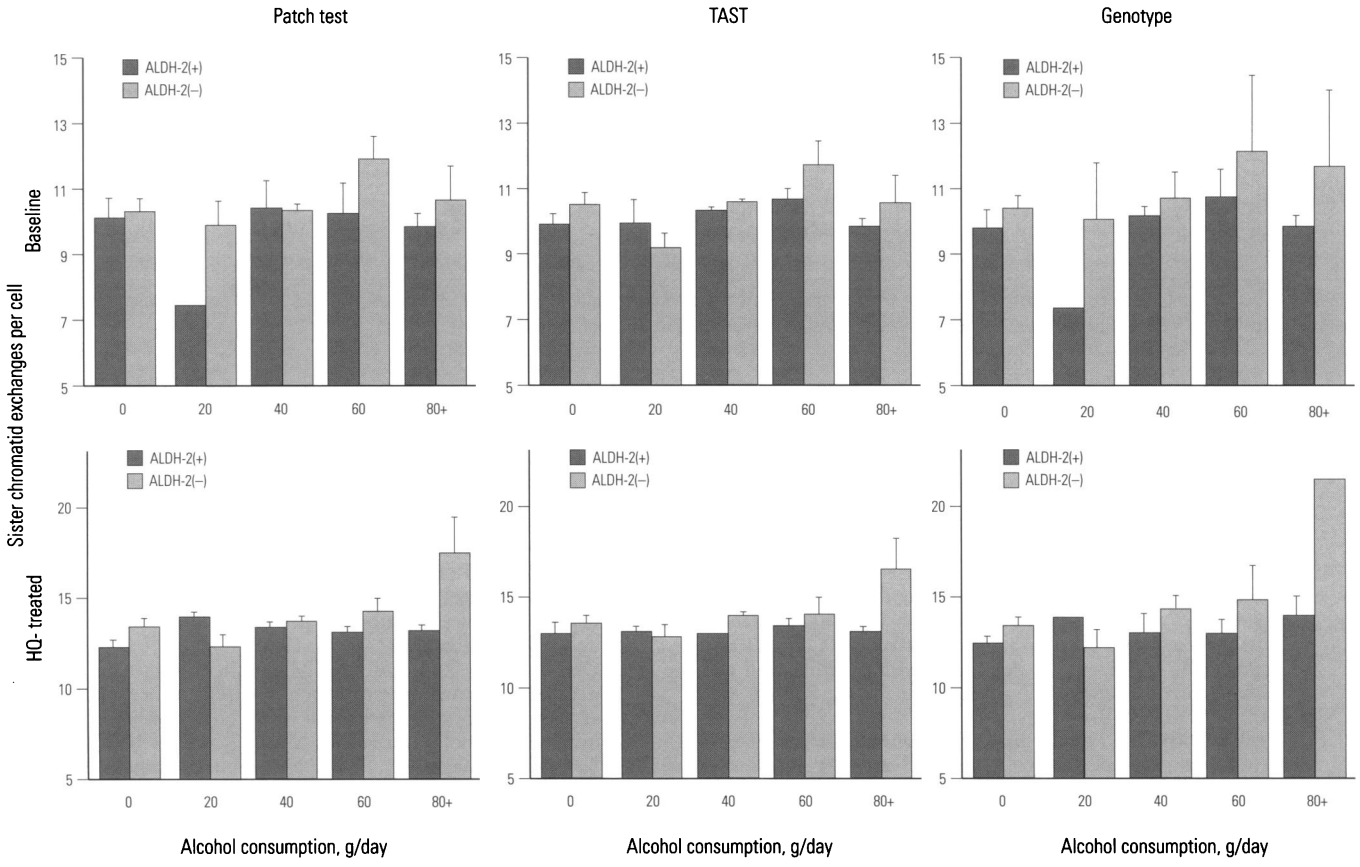


**Figure 1.** Enhanced frequencies of baseline (top row) and hydroquinone-induced (bottom row) sister chromatid exchanges in peripheral lymphocytes from ALDH2-deficient and nondeficient individuals.

**Table 4.** Effects of alcohol consumption on the induction of sister chromatid exchanges in lymphocytes based on ALDH2 status and controlled for smoking.

| | Patch test | | | TAST | | | Genotype | | |
|---|---|---|---|---|---|---|---|---|---|
| Alcohol consumption | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | |
| Almost every day | | | | | | | | | |
| Smoker | 9.58 ± 1.66 (23)[a] | 10.96 ± 2.14 (13) | $p=0.038$[b]* | 10.28 ± 1.30 (22) | 11.61 ± 2.05 (17) | $p=0.028$* | 9.44 ± 1.35 (27) | 11.84 ± 2.17 (10) | $p=0.007$* |
| Nonsmoker | 9.29 ± 1.24 (13) | 10.16 ± 1.76 (8) | $p=0.196$ | 9.82 ± 0.99 (15) | 10.93 ± 1.96 (8) | $p=0.083$ | 9.50 ± 1.44 (16) | 10.27 ± 1.74 (5) | $p=0.328$ |
| Several times per week or never | | | | | | | | | |
| Smoker | 10.46 ± 1.15 (6) | 9.00 ± 1.98 (18) | $p=0.129$ | 12.15 ± 1.37 (5) | 10.31 ± 1.53 (20) | $p=0.036$* | 11.60 ± 0.62 (4) | 9.09 ± 1.93 (18) | $p=0.000$* |
| Nonsmoker | 9.49 ± 1.33 (12) | 9.23 ± 1.98 (18) | $p=0.692$ | 9.35 ± 1.33 (12) | 9.89 ± 1.65 (20) | $p=0.340$ | 8.74 ± 1.50 (11) | 9.67 ± 1.79 (19) | $p=0.158$ |
| | $p=0.147$ | $p=0.757$ | | $p=0.006$* | $p=0.408$* | | $p=0.000$* | $p=0.351$* | |

Smoker's data was controlled on the assumption that one sister chromated exchange might be induced per cell per 20 cigarettes/smoker/day. [a]Values in parentheses = *n*. [b]Determined by *t*-test. *Denotes statistically significant values.

**Table 5.** Effects of alcohol consumption on the induction of sister chromatid exchanges in hydroquinone-treated[a] lymphocytes based on ALDH2 status and controlled for smoking.

| | Patch test | | | TAST | | | Genotype | | |
|---|---|---|---|---|---|---|---|---|---|
| Alcohol consumption | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | | ALDH2 (+) | ALDH2 (–) | |
| Almost every day | | | | | | | | | |
| Smoker | 12.58 ± 1.80 (20)[b] | 14.24 ± 2.03 (10) | $p=0.029$[c]* | 13.56 ± 1.45 (19) | 14.74 ± 2.05 (14) | $p=0.093$ | 12.35 ± 1.37 (24) | 15.47 ± 2.09 (7) | $p=0.006$* |
| Nonsmoker | 12.53 ± 2.03 (10) | 13.72 ± 3.63 (5) | $p=0.423$ | 12.35 ± 0.87 (9) | 15.28 ± 3.81 (6) | $p=0.041$* | 12.39 ± 1.86 (12) | 14.72 ± 4.74 (3) | $p=0.176$ |
| Several times per week or never | | | | | | | | | |
| Smoker | 12.23 ± 0.81 (4) | 12.00 ± 2.63 (10) | $p=0.873$ | 14.53 ± 0.91 (4) | 13.41 ± 1.77 (14) | $p=0.244$ | 12.01 ± 0.68 (4) | 12.08 ± 2.50 (11) | $p=0.958$ |
| Nonsmoker | 12.28 ± 1.39 (8) | 12.43 ± 1.69 (13) | $p=0.835$ | 11.96 ± 0.91 (10) | 13.31 ± 1.51 (23) | $p=0.015$* | 11.86 ± 1.52 (10) | 12.85 ± 1.49 (11) | $p=0.149$ |
| | $p=0.940$ | $p=0.636$ | | $p=0.004$* | $p=0.883$* | | $p=0.853$ | $p=0.394$ | |

Smoker's data was controlled on the assumption that one sister chromated exchange might be induced per cell per 20 cigarettes/smoker/day. [a]Hydroquinone treatment was $5 \times 10^{-5}$ M for 72 hr. [b]Values in parentheses = *n*. [c]Determined by *t*-test. *Denotes statistically significant values.

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