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

Kanehisa Morimoto and Tatsuya Takeshita

Department of Hygiene and Preventive Medicine, Osaka University School of Medicine, Osaka, Japan

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 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'-CCACCTCACCAGTTTTCTCTT) contained the substitution of an adenine by a thymine at the underlined portion in order to create a Ksp6321 recognition site (5'-CTCTT) 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 Ksp6321 (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 20 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.).

---

**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.

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

---

This paper was presented at the 2nd International Conference on Environmental Mutagens in Human Populations held 20–25 August 1995 in Prague, Czech Republic. Manuscript received 22 November 1995; manuscript accepted 28 November 1995.

We thank H. Ogura for her excellent technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

Address correspondence to Prof. Kanehisa Morimoto, Department of Hygiene and Preventive Medicine, Osaka University School of Medicine, Yamada-oka, Suita, Osaka 565, Japan. Telephone: +81-6-879-3920. Fax: +81-6-879-3929. E-mail: morimoto@envi.med.osaka-u.ac.jp

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'-GTTTTCAGTGTATG) or ALDH2\(^2\) allele (5'-GTTTTCAGTTAATGTATG). The membranes were washed three times at room temperature, washed with 1\(\times\)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\(\times\)10\(^7\)/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 \(\mu\)M 5-bromo-2'-deoxyuridine (BrdUrd). Proliferation was initiated by adding 3% phytohemagglutinin (PHA-M). Colcemid (2\(\times\)10\(^{-7}\) 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 (\(x^2 = 0.062\) 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.

| Alcohol consumption | Number (+) (age)\(^a\) | Number (-) (age)\(^b\) |
|---------------------|---------------------|---------------------|
| 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 \(x^2\)-test (\(p < 0.001\)). \(^a\)ALDH2\(^1/ALDH2\(^1\), \(^b\)ALDH2\(^2/ALDH2\(^2\) or ALDH2\(^2/ALDH2\(^2\). \(^\text{Mean} \pm \text{SD}\).

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 non-deficient 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 profuse 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 | Genotype |
|---------------------|------------|-----------|
|                     | ALDH2 (+)  | ALDH2 (−) | ALDH2 (+)  | ALDH2 (−) |
| Almost every day    | 10.29± 1.61 | 11.44± 1.99 | 10.18± 1.37 | 11.44± 1.98 | 10.17± 1.36 | 12.34± 2.03 |
|                     | (38)*      | (21)       | (41)        | (26)       | (43)        | (15)       |
| Several times per week | 10.14± 1.41 | 9.92± 1.55  | 10.08± 1.83  | 10.04± 1.45 | 9.96± 1.86 | 10.11± 1.46 |
| or never            | (18)       | (34)       | (18)        | (41)       | (15)        | (38)       |
| Alcohol consumption | p=0.020*   | p=0.630    | p=0.913     | p=0.759    |
|                     | p=0.003*   | p=0.0001*  | p=0.003*    |

Values in parentheses = n. *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.

| Alcohol consumption | Patch test | TAST | Genotype |
|---------------------|------------|------|----------|
|                     | ALDH2 (+)  | ALDH2 (−) | ALDH2 (+)  | ALDH2 (−) |
| Almost every day    | 13.40± 1.79 | 14.98± 2.68 | 13.14± 1.33 | 14.94± 2.76 | 13.11± 1.44 | 16.50± 2.70 |
|                     | (30)*      | (15)   | (22)      | (21)       | (36)        | (10)       |
| Several times per week | 12.59± 1.25 | 13.18± 1.66 | 12.68± 1.44 | 13.25± 1.57 | 12.39± 1.27 | 13.36± 1.57 |
| or never            | (12)       | (24)   | (15)      | (28)       | (14)        | (23)       |
| Alcohol consumption | p=0.021*   | p=0.281 | p=0.254   | p=0.059    |
|                     | p=0.005*   | p=0.017* | p=0.111   |

Hydroquinone treatment was 5×10^{-6} M for 72 hr. *Values in parentheses = n. *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.

| Alcohol consumption | ALDH2 (+) ALDH2 (-) | ALDH2 (+) ALDH2 (-) | Genotype |
|---------------------|----------------------|----------------------|----------|
| Almost every day    | Patch test           | TAST                 | Genotype |
| Smoker              |                      |                      |          |
| Alcohol consumption |                      |                      |          |
| Nonsmoker           |                      |                      |          |
| Smoker              |                      |                      |          |
| Nonsmoker           |                      |                      |          |

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

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

| Alcohol consumption | ALDH2 (+) ALDH2 (-) | ALDH2 (+) ALDH2 (-) | Genotype |
|---------------------|----------------------|----------------------|----------|
| Almost every day    | Patch test           | TAST                 | Genotype |
| Smoker              |                      |                      |          |
| Alcohol consumption |                      |                      |          |
| Nonsmoker           |                      |                      |          |
| Smoker              |                      |                      |          |
| Nonsmoker           |                      |                      |          |

Smoker’s data was controlled on the assumption that one sister chromatid exchange might be induced per cell per 20 cigarettes/smoker/day. *Hydroquinone treatment was 5×10⁻⁶ M for 72 hr. *Values in parentheses = n. *Determined by t-test. *Denotes statistically significant values.

REFERENCES

1. Berkman LF, Breslow L. Health and Ways of Living. The Alameda County Study. New York, Oxford:Oxford University Press, 1983.
2. Morimoto K. Lifestyles and health situations: importance of genetic health and quality of life. Position paper for the Workshop on Social Learning and Coping Options. Copenhagen:World Health Organization/EURO Office, 1986.
3. Hagihara A, Morimoto K. Personal health practices and attitudes toward nonsmokers’ legal rights in Japan. Soc Sci Med 33:717–721 (1991).
4. Kusaka Y, Kondou H, Morimoto K. Healthy lifestyles are associated with higher natural killer cell activity. Prev Med 21:602–614 (1992).
5. Bohlke JU, Singh S, Goedde HW. Cytogenetic effects of acetaldehyde in lymphocytes of Germans and Japanese: SCE, clastogenic activity, and cell cycle delay. Human Genet 63:285–289 (1983).
6. Hedner K, Wadstein J, Mitelman F. Increased sister chromatid exchange frequency in chronic alcoholic users. Hereditas 101:265–266 (1984).
7. Obe G, Ristow H. Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. Mutat Res 56:211–213 (1977).
8. Obe G, Ristow HJ, Herha J. Chromosomal damage by alcohol in vitro and in vivo. In: Alcohol Intoxication and Withdrawal: Experimental Studies. Vol IIIA (Gross MM, ed). New York:Plenum Press, 1977;47–70.
9. Obe G, Gobel D, Engeln H, Herha J, Natarajan AT. Chromosomal aberrations in peripheral lymphocytes of alcoholics. Mutat Res 73:377–386 (1980).
10. Obe G, Jonas R, Schmidt S. Metabolism of ethanol in vitro produces a compound which induces sister-chromatid exchanges in human peripheral lymphocytes in vitro: acetaldehyde but not ethanol is mutagenic. Mutat Res 174:47–51 (1986).
11. Bosron WF, Magnes LJ, Li TK. Human liver alcohol dehydrogenase: ADH Indianapolis results from the genetic polymorphism at the ADH2 gene locus. Biochim Genet 21:735–744 (1983).
12. Harada S, Agarwal DP, Goedde HW. Aldehyde dehydrogenase polymorphism and alcohol metabolism in alcoholics. Alcohol 2:391–392 (1985).
13. Edwards JA, Price-Evans DA. Ethanol metabolism in subjects possessing typical and atypical liver alcohol dehydrogenase. Clin Pharmacol Ther 8:824–829 (1967).
14. Agarwal DP, Goedde HW. Alcohol metabolism, alcohol intolerance, and alcoholism. Berlin, Heidelberg:Springer-Verlag, 1990.
15. Harada S, Agarwal DP, Goedde HW. Aldehyde dehydrogenase deficiency as cause of facial flushing reaction to alcohol in Japanese [letter]. Lancet 2:982 (1981).

16. Goedde HW, Singh S, Agarwal DP, Fritze G, Stapel K, Paik YK. Genotyping of mitochondrial aldehyde dehydrogenase in blood samples using allele-specific oligonucleotide: comparison with phenotyping in hair roots. Hum Genet 81:305–307 (1989).

17. Muramatsu T, Higuchi S, Shigemori K, Saito M, Sasaki M, Harada S, Shigeta Y, Yamada K, Muraoka H, Takagi S, Maruyama K, Kono H. Ethanol Patch test—a simple and sensitive method for identifying ALDH phenotype. Alcohol Clin Exp Res 13:229–231 (1989).

18. Morimoto K, Wolff S. Cell cycle kinetics in human lymphocyte cultures. Nature 288:604–606 (1980).

19. Takeda T, Morimoto K, Mao X, Hashimoto T, Furuyama J. Characterization of the three genotypes of low Km aldehyde dehydrogenase in a Japanese population. Hum Genet 94:217–223 (1994).

20. Morimoto K, Wolff S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 40:1189–1193 (1980).

21. Morimoto K. Induction of sister-chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res 43:1330–1334 (1983).

22. Takeda T, Morimoto K, Mao X, Hashimoto T, Furuyama J. Phenotypic differences in low Km aldehyde dehydrogenase in Japanese workers [letter]. Lancet 341:837–838 (1993).

23. Morimoto K, Wolff S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 40:1189–1193 (1980).

24. Morimoto K, Miura K, Kaneko T, Iijima K, Koizumi A. Human health situation and chromosomal alterations: sister chromatid exchange frequency in lymphocytes from passive smokers and patients with hereditary disease. In: Sister Chromatid Exchanges: Genetic Toxicology and Human Studies (Tice R, Hollaender A, Lambert B, Morimoto K, eds). New York: Plenum Press, 1984:801–812.