Characterization of the three genotypes of low $K_m$ aldehyde dehydrogenase in a Japanese population

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Abstract A deficiency in low $K_m$ aldehyde dehydrogenase (ALDH2) is regarded as the main factor responsible for “Oriental flushing” and other symptoms due to alcohol sensitivity. In this study, the relationship of the ALDH2 genotype to alcohol-associated symptoms and drinking behavior was investigated in 524 Japanese workers, using a new, rapid, and nonisotopic polymerase chain reaction (PCR) method. Differences in the frequency of alcohol-associated manifestations between the normal homozygote and the other deficient types were apparent. In addition, among the ALDH2-deficient individuals, the atypical homozygote was obviously more hypersensitive to alcohol than the heterozygote, judging from the frequency of flushing or other drinking-associated manifestations with a small dose of alcohol. Drinking frequency also apparently decreased in the following order: typical homozygote, heterozygote, atypical homozygote. Similarly, mean amounts of alcohol consumption also decreased in the same order, although considerable variation existed within the typical homozygote and the heterozygote group. In contrast, neither the manifestations nor the drinking behavior were, in general, influenced by polymorphism of the alcohol dehydrogenase $\beta$-subunit (ADH2) gene in males. These findings further indicate the important contribution of the ALDH2 genotype to alcohol sensitivity in Orientals.

Introduction

Low $K_m$ aldehyde dehydrogenase (ALDH2), mainly located in mitochondria (Meier-Tackmann et al. 1988), is regarded as being responsible for the oxidation of most of the acetaldehyde generated in alcohol metabolism (Agarwal and Goedde 1990). The “Oriental flushing” and other symptoms related to alcohol hypersensitivity in the Oriental population (Wolff 1972) are mainly due to a deficiency in this enzyme (Goedde et al. 1979; Harada et al. 1981). Furthermore, ALDH2 deficiency plays a protective role against alcoholism (Harada et al. 1982; Thomasson et al. 1991). It is important to investigate relationships between this polymorphism and alcohol-related health problems such as liver damage or hypertension.

Human ALDH2 cDNA has been cloned and a single point mutation in exon 12 of the ALDH2 gene has been found in those with defective ALDH2 (Hsu et al. 1983), corresponding to an amino acid substitution in the defective enzyme (Yoshida et al. 1984). The atypical allele (ALDH2$^2$) is dominant over the typical allele (ALDH2$^1$; Crabb et al. 1989; Goedde et al. 1989), and complete agreement between the ALDH2 genotype and phenotype has been reported (Crabb et al. 1989; Goedde et al. 1989; Singh et al. 1989). However, extensive characterization of the three ALDH2 genotypes has not been reported. Considerable differences between the heterozygote and the atypical homozygote with regard to blood acetaldehyde levels after drinking a small amount of alcohol (Enomoto et al. 1991b) suggest a phenotypic difference between the two genotypes.

In the current study, the ALDH2 genotypes were determined and their association with alcohol-related symptoms and drinking behavior were investigated in a large sample of Japanese workers. For this purpose, we devised a simple, nonisotopic polymerase chain reaction (PCR) method to detect the ALDH2 genotype. The results indicated that each of the three ALDH2 genotypes confers distinct phenotypic characteristics and drinking styles.

The atypical allele of the alcohol dehydrogenase $\beta$-subunit (ADH2) gene is also highly prevalent among Orientals (Goedde et al. 1992) and the atypical enzyme has a high $V_{max}$ (Yoshida et al. 1981; Yin et al. 1984). The relevance of this polymorphism to alcohol-related health problems has been indicated (Thomasson et al. 1991).
Hence, we also examined the effects of the ADH2 genotype on alcohol sensitivity and drinking behavior in a subgroup of the male subjects.

Materials and methods

Determination of ALDH2 genotypes

Blood samples (2-4 ml) were obtained with permission, from 424 male and 100 female workers (aged 38.8 ± 11.4 and 33.9 ± 13.3 years (means ± SD), respectively) in a metal plant with 649 workers in Japan. All subjects were nonalcoholic 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, Calif.). Exon 12 of the ALDH2 gene was amplified by 30-35 cycles of PCR (1 min at 94°C, 10 s at 52°C, and 30 s at 72°C) in a Perkin-Elmer Cetus (Norwalk, Conn.) Thermal Cycler. Amplification primers were as previously reported (Goedde et al. 1989), except that one primer (5'-CCACACTCACGTTCACCTTTCCCTTT) contained the substitution of an adenine by a thymine at the underlined portion 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, 2-3 units of Ksp632I (Boehringer Mannheim) and the reaction buffer was incubated at 37°C for 3-6 h, 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 the number of amplification cycles was 40. Amplification primers were the same as described previously (Goedde et al. 1989). PCR products (5-10 µl) were treated with alkali and transferred to nylon membranes, which were then baked and hybridized overnight with 32P-ATP (Amersham) labeled probe, for either ALDH21 (5'-GTTTTCACTTCAGTGTAAG; ALDH21) or ALDH22 allele (5'-GTTTTCACTTTAGTGTATG). The membranes were first washed three times at room temperature, then washed with 1 x SSC containing 0.1% sodium dodecylsulfate (SDS) for 30 min at 48°C, and exposed to X-ray film overnight.

Determination of ADH1 genotypes

A subgroup (n = 151) was randomly selected from each ALDH2 genotype group of the male subjects for analysis of the ADH1 genotypes. The genotypes were determined according to the method described previously (Xu et al. 1988), using PCR and HaeIII digestion.

Characterization of phenotypes

All subjects completed a questionnaire, including the frequency of alcohol-associated symptoms with the usual dose or with a quarter glass of beer, and drinking habits, before they underwent the patch test. All of the questions on alcohol-related symptoms described in the previous report (Yamada et al. 1988) were included in the present study.

Statistical methods

Alcohol-associated symptoms and drinking frequency were compared among the ALDH2 genotypes, using either the chi-square test or Fisher’s exact test. Differences in the mean amounts of alcohol consumption among the genotypes were tested by Wilcoxon’s test because the values were not normally distributed except in the male typical homozygotes.

Results

PCR amplification using a mutated primer yielded sufficient products, of which 135 base pairs (bp) were cut into 112- and 23-bp fragments by the restriction enzyme Ksp632I, only in the typical allele. As shown in Fig. 1, 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 (ALDH21/ALDH21), 38 of the heterozygotes (ALDH21/ALDH22), and 16 of the atypical homozygotes (ALDH22/ALDH22).

The frequencies of the three ALDH2 genotypes in the subjects are shown in Table 1. Gene frequencies of the typical and atypical alleles calculated from the genotype frequencies were 0.743 and 0.267 for males and 0.790 and 0.210 for females. Deviation from Hardy-Weinberg’s prediction was not statistically significant in either sex ($\chi^2 = 0.062$ and 0.126, $df = 1, P > 0.5$). Differences in the allele frequencies between males and females were not significant at the chi-square test ($\chi^2 = 1.679, df = 1, P > 0.10$).

The frequency of alcohol-associated symptoms was compared among the three ALDH2 genotypes (Tables 2, 3). A quarter glass of beer was assumed to contain 40 ml of beer. Mean body weights (kg) for the typical homozygote, the heterozygote, and the atypical homozygote were calculated to be 62.9, 63.5, and 63.9 for the males; 50.9, 50.2, and 51.0 for the females, respectively. Hence, the equivalent dose of pure ethanol (ml) per kg body weight for a quarter glass of beer was calculated to be 0.032, 0.031, and 0.031 for the males; 0.039, 0.040, and 0.039 for the females, respectively.