Am. J. Hum. Genet. 43:741–743, 1988

Frequency of the Atypical Aldehyde Dehydrogenase-2 Gene ($ALDH_2^2$) in Japanese and Caucasians

Akitaka Shibuya and Akira Yoshida

Beckman Research Institute of the City of Hope, Duarte, CA

Summary

All Caucasians have two major aldehyde dehydrogenase isozymes—i.e., the cytosolic $ALDH_1$ and the mitochondrial $ALDH_2$—while approximately 50% of Orientals are atypical and lack the catalytically active $ALDH_2$ in their tissues. The atypical $ALDH_2^2$ gene has a nucleotide base change and produces the defective $ALDH_2^2$ protein, which has a Glu→Lys substitution at the 14th position from the COOH-terminal (Yoshida et al. 1984; Hsu et al. 1985). With the use of a pair of synthetic oligonucleotides—one complementary to the usual $ALDH_2^1$ and the other complementary to the atypical $ALDH_2^2$—genotypes of 49 unrelated Japanese individuals and 12 Caucasians were determined. The frequency of the atypical $ALDH_2^2$ allele was found to be .35 in the Japanese samples examined. The atypical $ALDH_2^2$ gene was not found in the Caucasians.

Introduction

Approximately 50% of Japanese and other Orientals have the atypical aldehyde dehydrogenase-2 (mitochondrial isozyme) phenotype, which lacks or severely diminishes the enzyme activity (Goedde et al. 1979; Teng 1981). $ALDH_2$ has a low K_m for acetaldehyde and is considered to play a major role in alcohol detoxification. Thus, the atypical $ALDH_2$ phenotype has been implicated in the high incidence of acute alcohol intoxication ("alcohol flushing") in Orientals (Goedde et al. 1979; Harada et al. 1980).

The atypical $ALDH_2$ allele has the nucleotide base transition ($\frac{G\rightarrow A}{C\rightarrow T}$) in its exon 12, and the gene product, i.e., atypical $ALDH_2^2$ protein, contains a Glu→Lys substitution at the 14th position from the COOH-terminal (Yoshida et al. 1984; Hsu et al. 1985, 1987, 1988). Utilizing a pair of allele-specific synthetic nucleotides, one complementary to the usual $ALDH_2^1$ and the other complementary to the atypical $ALDH_2^2$, we determined the $ALDH_2$ genotypes and the frequencies of the usual and atypical alleles.

Received March 22, 1988; revision received May 4, 1988.

Address for correspondence and reprints: Akira Yoshida, Ph.D., Department of Biochemical Genetics, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

0002-9297/88/4305-0021$02.00

Material and Methods

For genotype determination, DNAs were prepared from fresh whole blood by the established method (Blin and Stafford, 1976). The DNA samples (~10 μg each) were completely digested by *PstI*, and the restriction fragments were separated by agarose-gel electrophoresis. The first in-gel hybridization was carried out using the P_{32}-labeled oligonucleotide probe specific to the $ALDH_2^1$. The hybridized gel was treated with alkaline solution to remove the first probe, and the gel was rehybridized with the P_{32}-labeled oligonucleotide specific to the $ALDH_2^2$. The details of the procedures, including the conditions of agarose-gel electrophoresis, hybridization, washing, and rehybridization, have been described in a previous publication (Hsu et al. 1987).

Results and Discussion

DNA samples obtained from 49 unrelated Japanese individuals and from 12 Caucasians were examined. Examples of hybridization profiles of the DNA samples with the allele-specific oligonucleotide probes are shown in figure 1. The genotypes of the $ALDH_2$ locus, thus determined, are summarized in table 1. The frequency of the atypical $ALDH_2^2$ allele was found to be .35 in a total of 98 Japanese $ALDH_2$ loci examined, while it was zero in Caucasians.

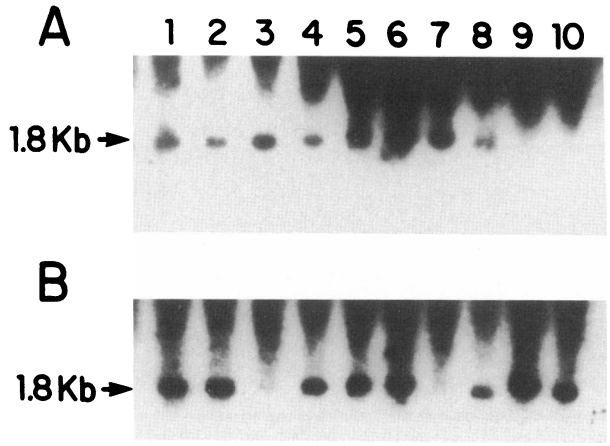

Figure 1 Hybridization of human genomic DNAs with allele-specific oligonucleotide probes. DNA samples were digested with *PstI*, electrophoresed in 0.9% agarose gel, and subjected to hybridization. *A*, Hybridization with probe-1 (specific for $ALDH_2^1$ allele) at 58 C for 20 h; four washes at 25 C for 30 min and two washes at stringent 58 C for 2 min. Probe-1: 3′ C·CGT·ATG·TGA·CTT·CAC·TTT·TG 5′. *B*, Hybridization with probe-2 (specific for $ALDH_2^2$ allele) at 50 C for 20 h; four washes at 25 C for 30 min and two washes at stringent 54 C for 2 min. Probe-2: 5′-G·GCA·TAC·ACT·AAA·GTG·AAA·AC 3′. Samples 3 and 7 are judged to be homozygous usual $ALDH_2^1/ALDH_2^1$; samples 1, 2, 4–6, and 8 are heterozygous atypical $ALDH_2^1/ALDH_2^2$; and samples 9 and 10 are homozygous atypical $ALDH_2^2/ALDH_2^2$.

$ALDH_2$ is a tetrameric enzyme. (Greenfield and Pietruszko 1977; Ikawa et al. 1983). Heterozygous $ALDH_2^1/ALDH_2^2$ subjects can produce the usual $ALDH_2^1$ subunit and the active homotetramer, although the quantity would be reduced. These subjects would be phenotyped as usual in the electrophoretic examination. On the basis of this assumption and the observed frequency (about 50%) of the atypical $ALDH_2$ phenotype (Goedde et al. 1979; Teng 1981), the frequency of the atypical $ALDH_2^2$ allele was estimated to be about .7 (Yoshida 1983). The present direct determination ($ALDH_2^2$ = .35) reveals that the previous estimation is incorrect. This finding implies that not only the atypical homotetramer (bbbb; b = product of the atypical $ALDH_2^2$ allele) but also the three hetero-tetramers (aaab, aabb, and abbb) are catalytically inactive (or far less active) and that only the usual homotetramer (aaaa; a = product of the usual $ALDH_2^1$ allele) exhibits enzyme activity. The amount of usual homotetramer in the heterozygous tissues would be only about 6% of that in the usual tissues, if the usual $ALDH_2^1$ and the atypical $ALDH_2^2$ genes produce an equal amount of proteins. The atypical subunit and the heterotetramers would be more labile in vivo, as suggested by a low content of $ALDH_2^2$ component in apparent homozygous atypical livers (Impraim et al. 1982; Ikawa et al. 1983). Thus, the heterozygous $ALDH_2^1/ALDH_2^2$ would be classified as atypical by enzyme electrophoresis (or isoelectric focusing) in most cases (Goedde et al. 1979; Teng 1981).

The present study indicates that the frequency of the $ALDH_2^2$ allele is about .35 in Japanese. The possibility of the existence of other variant allele(s) is not excluded in this study. Thus, another common variant allele, $ALDH_2^3$, which produces a defective subunit, could exist in Japanese. Assuming that (1) the frequencies are $ALDH_2^1$ = .30, $ALDH_2^2$ = .35, and $ALDH_2^3$ = .35, (2) subjects with $ALDH_2^1/ALDH_2^1$, $ALDH_2^1/ALDH_2^2$, and $ALDH_2^1/ALDH_2^3$ exhibit the enzyme activity, and (3) subjects with $ALDH_2^2/ALDH_2^2$, $ALDH_2^2/ALDH_2^3$, and $ALDH_2^3/ALDH_2^3$ lack the activity, the observed frequency of $ALDH_2$ enzyme deficiency (about 50%) can be explained by the three-allele model.

The two models, i.e., the two-allele model ($ALDH_2^1$ = .65 and $ALDH_2^2$ = .35) and the three-allele model ($ALDH_2^1$ = .30, $ALDH_2^2$ = 0.35, and $ALDH_2^3$ = .35) can be tested by examining the mode of inheritance of

Table 1

Genotypes of the *$ALDH_2$* Locus in Japanese and Caucasians

	Genotypes			Gene Frequency	
	$ALDH_2^1/ALDH_2^1$	$ALDH_2^1/ALDH_2^2$	$ALDH_2^2/ALDH_2^2$	$ALDH_2^1$	$ALDH_2^2$
Japanese (n = 49):					
Observed	21	22	6	.65	.35
Calculated[a]	20.7	22.3	6.0		
Caucasians (n = 12):					
Observed	12	0	0	1.0	0
Calculated[a]	12	0	0		

[a] Based on Hardy-Weinberg genetic equilibrium.

the flushing character. The two-allele model predicts that (*a*) if both parents are nonflushers (i.e., homozygous usual $ALDH_2^1/ALDH_2^1$) the offspring will be nonflushers and (*b*) if both parents are flushers (i.e., heterozygous $ALDH_2^1/ALDH_2^2$ or homozygous atypical $ALDH_2^2/ALDH^2$) the offspring could be flushers or nonflushers. The three-allele model predicts that all offspring of flushers should be flushers.

Acknowledgment

This work was supported by National Institutes of Health grant AA05763.

References

Blin, H., and D. W. Stafford. 1976. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acids Res. 3:2303–2308.

Goedde, H. W., S. Harada, and D. P. Agarwal. 1979. Racial differences in alcohol sensitivity: a new hypothesis. Hum. Genet. 51:331–334.

Greenfield, N. J., and R. Pietruszko. 1977. Two aldehyde dehydrogenases from human liver: isolation via affinity chromatography and characterization of the isozymes. Biochim. Biophys. Acta 483:35–45.

Harada, S., S. Misawa, D. P. Agarwal, and H. W. Goedde. 1980. Liver alcohol dehydrogenase and aldehyde dehydrogenase in Japanese: isozyme variation and its possible role in alcohol intoxication. Am. J. Hum. Genet. 32:8–15.

Hsu, L. C., R. E. Bendel, and A. Yoshida. 1987. Direct detection of usual and atypical alleles on the human aldehyde dehydrogenase-2 ($ALDH_2$) locus. Am. J. Hum. Genet. 41:996–1001.

———. 1988. Genomic structure of the human mitochondrial aldehyde dehydrogenase gene. Genomics 2:57–65.

Hsu, L. C., K. Tani, T. Fujiyoshi, K. Kurachi, and A. Yoshida. 1985. Cloning of cDNAs for human aldehyde dehydrogenase 1 and 2. Proc. Natl. Acad. Sci. USA 82:3771–3775.

Ikawa, M., C. C. Impraim, and A. Yoshida. 1983. Isolation and characterization of aldehyde dehydrogenase isozymes from usual and atypical human livers. J. Biol. Chem. 258:6282–6287.

Impraim, C., G. Wang, and A. Yoshida. 1982. Structural mutation in a major human aldehyde dehydrogenase results in loss of enzyme activity. Am. J. Hum. Genet. 34:837–841.

Teng, Y. S. 1981. Human liver aldehyde dehydrogenase in Chinese and Asiatic Indians: gene detection and the possible implication in alcohol metabolism. Biochem. Genet. 19: 107–113.

Yoshida, A. 1983. Differences in the enzymes involved in alcohol metabolism between Caucasians and Orientals. Pp. 245–261 *in* Biological and medical research. Vol. 8. Alan R. Liss, New York.

Yoshida, A., I.-Y. Huang, and M. Ikawa. 1984. Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. Proc. Natl. Acad. Sci. USA 81:258–261.