Aldehyde Dehydrogenase-2 Genotype Detection in Fingernails among Non-alcoholic Northeastern Thai Population and Derived Gene Frequency

Paiboon Mongconthawornchai[a,]*, Somsong Nanakorn[a], Atsushi Nishiyori[b]
[a] Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
[b] Department of Public Health, Kurume University School of Medicine, Kurume city, Japan.
* Corresponding author. Email: paimon@kku.ac.th

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ABSTRACT A genetic study on aldehyde dehydrogenase 2 (ALDH2) genotype was performed in a rural area of Khon Kaen province, northeastern Thailand. One hundred and twenty four fingernail specimens of probable non-alcoholics were obtained from unrelated male villagers 18-65 years old. The alcoholism screening test named "the Michigan Alcoholism Screening Test-Thai version (MAST-T)" was used for inclusion of probable-non-alcoholic subjects. Genomic DNA was extracted from clipped fingernails and ALDH2 genotypes were determined by PCR, *Ksp 632I*, digested and polyacrylamide gel electrophoresis. Most of the subjects (91.1%) were of the normal homozygous genotype (ALDH2*1/ALDH2*1), 8.9%, heterozygous genotype (ALDH2*1/ALDH2*2), and none of them were the mutant homozygous genotype (ALDH2*2/ALDH2*2). Gene frequencies of ALDH2*1 and ALDH2*2 alleles calculated from the genotype frequencies were 0.956 and 0.044, respectively. Deviation from the Hardy-Weinberg' s equilibrium was not statistically significant. The present study confirms the high frequency of ALDH2*1 gene (0.950-0.956) in non-alcoholic Thai population. Interestingly, the ALDH2*2 gene is not prevalent among Thai population compared with the Japanese and Chinese, though, they are of Mongoloid origin. The information on ALDH2 gene frequency of Oriental populations and Caucasoids were discussed.

KEYWORDS: ALDH2 genotype, gene/allele frequency, fingernails, Michigan Alcoholism Screening Test, Thais.

INTRODUCTION

Ethnic differences in sensitivity to alcohol are well recognized, particularly between Mongoloids and Caucasoids. A significantly greater percentage of Orientals respond to a mild dose of alcohol intake with marked adverse reactions such as facial flushing, increased heart rate, tachycardia, hot feeling in the stomach and muscle weakness.[1] The main pathway of ethanol metabolism occurs by alcohol dehydrogenase (ADH) oxidising ethanol to acetaldehyde, and aldehyde dehydrogenase (ALDH) converting acetaldehyde to acetate.[2] Two major isozymes of ALDH ie, ALDH1 and ALDH2 have been well characterized. ALDH1, which is mainly localized in the cytosol of liver cells, has a high affinity for acetaldehyde, while ALDH2, localized in the mitochondria, has a low affinity. A genetic polymorphism in the ALDH2 gene locus on the chromosome 12 encodes an inactive subunit with a point mutation corresponding to an amino acid substitution.[3] The mutant allele, ALDH2*2, is dominant over the normal allele (ALDH2*1); individuals who have this allele has little or no enzyme activity.[4] Subjects with the ALDH2*2 allele, both homozygous and heterozygous, experience more intense reactions to alcohol than subjects with only ALDH2*1.[5,6] These individuals are alcohol sensitive and have a markedly reduced risk to develop alcoholism and alcoholic liver diseases. Accordingly, there exist three genotypes, ie, ALDH2*1/ALDH 2*1, ALDH2*1/ALDH2*2, and ALDH2*2/ALDH2*2. Gene-geography studies have shown that Oriental populations of Mongoloid origin have high frequencies of the inactive ALDH2*2 allele (ALDH2*1/ALDH2*2 or ALDH2*2/ALDH2*2 genotypes), while this allele has been extremely rared in Caucasoids.[6,7] The present study is aimed at detection of ALDH2 genotype by using enzymatically amplified genomic DNA extracted from fingernails, and calculation of gene frequency among the northeastern Thai population, who are of Oriental or Mongoloid origins.

Materials and Methods

Collection of specimen

Unrelated male subjects aged between 18-65 years old living in rural villages of Khon Kaen province, northeastern Thailand were scheduled to be interviewed with the modified Michigan Alcoholism Screening Test-Thai version (MAST-T) to recruit probable non-alcoholic subjects as described elsewhere.[8] In sum, the MAST-T is a 23-item questionnaire, which was validated and yielded high sensitivity and specificity to screen alcoholics among the northeastern Thai population.[9] Subjects who scored less than eleven, which was a cutoff point on this MAST-T, were classified as probable non-alcoholics. Following the signed inform consents, one hundred and twenty-four subjects of probable non-alcoholics were selected. Then, clipped fingernails were properly collected for each selected subject and transported to the Kurume University School of Medicine Department of Public Health, Japan for determination of ALDH2 genotypes.

This study was approved by the Ethical Review Research Committee of the Ministry of Public Health, Thailand.

Detection of ALDH2 genotype

The ALDH2 genotype of each clipped fingernail was detected using PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). Genomic DNA was extracted from 20-30 mg of nail clippings with 300 μL of 4 mol / L guanidine thiocyanate, 0.5% sodium-N-lauroyl- sarcosinate, 25 mmol / L sodium citrate and 0.1 mol /L 2-mercapto-ethanol, according to a standard method for RNA extraction,[10] modified for DNA extraction from fingernails.[11] The denatured protein was removed by phenol/chloroform {saturated with TE buffer [10 mmol / L Tris-HCL (pH 8.0), 1 mmol / L EDTA (pH 8.0)]}, then the DNA was precipitated with ethanol and sodium chloride. After centrifugation, the genomic DNA pellet was dissolved in 30 μL of TE buffer diluted 1:10. One-fifteenth of the genomic DNA was subjected to 30 cycles (1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C)[12] in a *Techne PHC-3* thermal cycler with standard amplification primers designed by Goedde *et al*[13] and Takeshita *et al*[14] as following: forward primer (5'-CAAATTACAGGGTC AACTGCT) is the same as previous reported by Goedde *et al*[13], reverse primer (5'-CCACACTCACA GTTTTCTCTT).[14] The latter contained the substitution of an adenine by a thymine at the underlined portion to create a *Ksp 632I* recognition site (5'-CTCTTC). The PCR products (4-8 μL) were digested with 1 unit of *Ksp 632I* (Boehringer Mannheim) and incubated at 37°C for at least 4 hours to cut solely the 135 bp DNA fragments of normal allele into fragments of 112 bp and 23 bp. The mutant allele could not be cut with this enzyme. Then, the fragments were separated by electrophoresis in 8% BIS-acrylamide gel, visualized by ethidium bromide, staining and photographed under UV light with a polaroid film *T 667 ISO 3000 (*Polaroid Corporation, Cambridge, Massachusetts, USA).

Results and Discussion

As shown in Fig 1, the three genotypes of ALDH2 were distinguishable. Genotype frequencies were as follows: 91.1%, the normal or typical homozygous genotype (ALDH2*1/ALDH2*1), 8.9%, the heterozygous genotype (ALDH2*1/ALDH2*2), and none the homozygous mutant or atypical genotype (ALDH2*2/ALDH2*2), as shown in Table 1. Gene frequencies of ALDH2*1 and ALDH2*2 alleles derived from genotype frequencies were of 0.956 and 0.044, respectively (Table 1). Deviation from the Hardy-Weinberg's equilibrium[15] was not statistically significant (χ^2=0.272, df=1, p=0.603).

Table 2 shows distribution of ALDH2 genotypes and gene frequencies among various populations of Mongoloid and Caucasoid origin. The determined gene frequency in the present study was consistent with the previous data of the northern Thai population (0.950 for ALDH2*1 allele, and 0.05 for ALDH2*2 allele) studied by Goedde et al.[7]

Interestingly, the ALDH2*2 allele frequencies of both the northern and the northeastern Thais seem

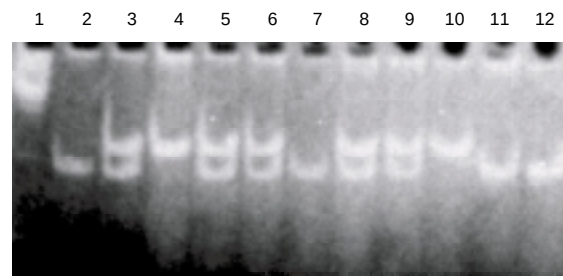

Fig 1. *ALDH2 genotypes obtained by electrophoresis, 8% Bis-acrylamide gel, visualized by ethidium bromide staining. Smaller fragments (112 bp) correspond to the typical allele (ALDH2*1), and larger fragments (135 bp) to the atypical allele (ALDH2*2).*

Lanes 2, 7, 11, 12 show the normal or typical homozygote (ALDH2*1/ ALDH 2*1);

Lanes 3, 5, 6, 8, 9, the heterozygote (ALDH2*1/ALDH 2*2); and Lane 4, and 10, the mutant or atypical homozygote (ALDH2*2/ALDH 2*2).

to be similar to that found among Malays[7] (0.034), Taiwanese aborigines[17] (0.02), and Filipinos[7] (0.006). It was concluded by Chen et al[17] that the similar low frequency of ALDH2*2 allele among Taiwanese and Malays are consistent with the contention that Taiwanese aborigines are descended from Malay-Polynesian stock. The study by Goedde et al[7] revealed a low frequency of ALDH2*2 allele among some populations of Caucasoid origin ie, Indians (0.02), and Hungarians (0.013). The low frequency of ALDH2*2 gene (0.044-0.05) among Thai populations is different from that of prevalence among Mongoloids such as Japanese (0.236-0.271),[7, 14, 18-19] Chinese (0.30, 0.25).[16, 22] It implies that fewer Thais will probably be sensitive to alcohol, as compared with Japanese, who have a relatively high ALDH2*2 allele frequency. It can be concluded that the healthy and non-alcoholic Thai populations from the north and northeastern regions, respectively, possess an ALDH2 gene frequency different from that of Japanese or some populations of Chinese origin.

Table 1. ALDH2 genotype, and derived gene frequencies among northeastern Thais.

ALDH2 genotype	Number observed	Genotype frequency	Number expected
ALDH2*1/ALDH2*1	113	91.129	113.328
ALDH2*1/ALDH2*2	11	8.871	10.432
ALDH2*2/ALDH2*2	0	0	0.24
Total	**124**	**100**	**124**

Gene frequency: ALDH2*1 = 0.956
ALDH2*2 = 0.044
$\chi^2 = 0.272$, df = 1, $p = 0.603$

Table 2. Distribution of ALDH2 genotype and gene frequency among various populations of Mongoloids and Caucasoids origin.

Subjects	n	Genotype frequency			Gene frequency	
		ALDH2*1/*1	ALDH2*1/*2	ALDH2*2/*2	ALDH2*1	ALDH2*2
Mongoloids:						
Thais {Northeast}*	124	113	11	0	0.956	0.044
Thais {North} (7)	111	100	11	0	0.95	0.05
Filipinos (7)	86	85	1	0	0.994	0.006
Malays (7)	73	68	5	0	0.966	0.034
Koreans (7)	218	156	58	4	0.849	0.151
Chinese (7)	132	92	38	2	0.841	0.159
Chinese (16)	50	26	18	6	0.7	0.3
Taiwanese aborigine (17)	58	56	2	0	0.98	0.02
Japanese(7)	53	29	23	1	0.764	0.236
Japanese (18)	58	32	21	5	0.73	0.27
Japanese (14)	424	235	160	29	0.743	0.257
Japanese (19)	129	70	48	11	0.729	0.271
Caucasoids:						
Germans (7)	193	193	0	0	1	
Swedes (7)	99	99	0	0	1	
Hungarians (7)	117	114	3	0	0.987	0.013
Indians (7)	179	173	5	1	0.98	0.02

* present study
(7) Goedde et al 1992
(14) Takeshita et al 1994
(16) Thomasson et al 1991
(17) Chen et al 1998.
(18) Yamamoto et al 1993
(19) Yuasa et al 1997

The present study was performed in the selected non-alcoholic group, which was more likely to maintain a higher ALDH2*2 allele frequency than that in the alcoholic group, as shown in previous studies. Higuchi et al[20] found ALDH2*2 allele frequencies as 0.243 (n=461) and 0.061 (n=655) in Japanese non-alcoholics and alcoholics, respectively, while Maezawa et al[21] found 0.267 (n=60) and 0.114 (n=96), respectively. Chinese non-alcoholics and alcoholics had ALDH2*2 allele frequencies of 0.25 (n=105) and 0.05 (n=32), respectively.[22] Many studies[4, 13, 16] have reported that the genotypes of ALDH2*1/2*2 and ALDH2*2/2*2 phenotypically correspond to the deficiency of ALDH2 enzyme activity, and are found at lower frequency in alcoholics. These previous studies confirm that there is a significant difference in ALDH2 allele frequencies between non-alcoholics and alcoholics of both Japanese and Chinese origin, so ALDH2*1 is one of the genetic factors affecting the risk of developing alcoholism. In other words, these facts support the hypothesis that ALDH2*2 is a protective factor for alcoholism among Japanese and Chinese, as suggested by Goedde et al.[7] However, this hypothesis could not be generalized for other Asians who are of Mongoloid origin, eg. Thais, Filipinos and Malays, as they possess lower ALDH2*2 allele frequencies than that of Japanese and Chinese.

So far there has been no study on ALDH2 gene frequencies among non-alcoholic *vs.* alcoholic Thais. Although, there was one report concerning ALDH2 allele frequencies among healthy Thais[7], this information cannot clarify whether genetic factors affect the risk of developing alcoholism in Thais. Accordingly, the determination of ALDH2 allele frequencies among non-alcoholics Thais is worthy, though the comparison of ALDH2 allele frequencies among non-alcoholics and alcoholics is required to elucidate this point.

A study by Thomasson et al[23] on the relationship of the ALDH2, ADH2, and ADH3 alleles to the alcohol metabolic rate, alcohol flush intensity, and inheritance of the alleles of these loci in Chinese alcoholics versus non-alcoholics, suggested that both the ALDH2 and the ADH2 genotypes exert an influence on alcohol metabolic rate, alcohol flush reaction, and susceptibility to developing alcoholism. We further suggest an investigation of relationships between ALDH2*2 and ADH2*2 alleles with alcohol adverse reaction and amount of blood acetaldehyde, which is mandatory for exploring what kinds of alcohol-metabolizing enzymes do affect the alcohol adverse reaction or may be a protective factor for alcoholism among Thais. It also would be of value to compare pattern of drinking in a healthy population with a view to detecting an association between the patterns of alcohol-metabolizing enzymes and different aspects of drinking behavior.

The clipped fingernails were used for detecting ALDH2 genotypes instead of blood or hair roots for two reasons. First, the improved method of ALDH2 genotyping using fingernails has been proved to yield consistent genotyping compared with blood specimens.[11-12] Secondly, collecting fingernails from a general healthy population is more safe and feasible compared with the collection of blood or hair roots.

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