Genotype-Phenotype Agreement of Aldehyde Dehydrogenase 2 in 120 Healthy Japanese

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Summary: An individual genotype of aldehyde dehydrogenase 2 was observed using nail clippings by an improved method consisting of DNA extraction with guanidium thiocyanate, genomic DNA amplification by non-isotopic polymerase chain reaction, and gel electrophoresis of the DNA fragment. The results were consistent with those obtained using the corresponding peripheral blood samples. The individual phenotype was also determined by the Tokyo-University ALDH2-Phenotype Screening Test. Both types of ALDH2 were observed in 120 healthy Japanese and 48% were the typical or normal homozygote, 43% were the heterozygote, and 9% were the atypical or mutant homozygote of ALDH2. Among 57 individuals of both sexes with the typical homozygote, 88% were the phenotype of a non-flusher. Similarly 76% of the 63 heterozygote/atypical homozygotes had the phenotype of a flusher.

Key words: genotype — phenotype — aldehyde dehydrogenase 2 — Japanese — polymerase chain reaction

Introduction

Ingested alcohol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH), which is then metabolized to acetic acid by aldehyde dehydrogenase (ALDH). ALDH2 which has a low Km value is regarded as the critical ALDH isoenzyme that determines an individual's intolerance to alcohol. Genotyping or phenotyping of ALDH2 is essential for genetic, clinical, or epidemiological studies of alcohol-related diseases. Since it is not always feasible to collect blood samples; other biological materials, such as scraped mucous membranes, urine,

hair roots or fingernails can be used. Gene analysis using fingernail DNA has been reported (Kaneshige et al. 1992) but the proteinase K method is rather time consuming and may fail to extract DNA from manicured or smudgy nails as exemplified by Fig.1A. Phenotyping of an individual's ALDH2 can be performed by the Tokyo-University ALDH2-Phenotype Screening Test (TAST) which is a questionnaire consisting of 13 items (Yamada et al. 1988), and it may be used as an alternative measure for the genotype. Therefore, agreement between the two types is to be elucidated in human populations by race or other relevant factors.

Fig. 1. A gel electropherogram of one-tenth of the PCR products of a 135 bp DNA fragment containing the ALDH2 exon 12 from fingernails. The size marker is ϕ X174/Hae III (M). A: The DNA fragment was not detected in 3 manicured (lanes 1-3) and 3 smudgy nails (lanes 4-6) by the proteinase K method. Applying half of the same PCR products to the same electrophoresis also failed to yield a DNA fragment by the proteinase K method (data not shown). B: The DNA fragment was detected in the corresponding 3 manicured (lanes 1-3) and 3 smudgy nails (lanes 4-6) by the guanidium method.

The present paper describes an improved ALDH2 genotyping method using nail clippings that yields results consistent with peripheral blood samples, and that agrees with the corresponding phenotypes of healthy Japanese.

Materials and Methods

Nails were collected using an ordinal nail clipper which was cleaned with 70% ethanolic cotton pads. The nails were cut into pieces of approximately 1-2 $\times$ 5-10 mm, and 10-20 mg of the nail clippings were rinsed once with 1.0 N NaOH and twice with distilled water in a 1.5 ml micro test-tube. After adding 300 μ l of 4 M guanidium thiocyanate (Chomczynski and Sacchi, 1992), 0.5% sodium N-lau-

roylsarcosine, 25 mM sodium citrate and 0.1 M 2-mercaptoethanol, the sample was incubated at pH 7.0 at room temperature for 30 min. Nucleic acids were extracted with phenol/chloroform and precipitated with ethanol and sodium acetate. After centrifugation the pellet was washed in 70% ethanol and the dried pellet was dissolved in 20 μ l of distilled water. One-tenth of the genomic DNA was subjected to 30 cycles of amplification by polymerase chain reaction (PCR) (Takeshita et al. 1993). 5-15 μ l of the PCR products were digested by Ksp 632 I to solely cut the 135 bp DNA fragment of a typical homozygote containing ALDH2 exon 12 into fragments of 112 bp and 23 bp. 5 μ l of the PCR products were analyzed in 4% agarose gel, visualized by ethidium bromide staining and photographed under

UV light.

Although disposable nail clippers are preferable for sampling to prevent DNA contamination, the DNA contamination due to the use of a single nail clipper for collecting many nails was examined by processing homozygous nails after heterozygous nails using a single clipper being cleaned as stated above.

The recovery of DNA was determined as follows. 480 μ l of Tris-EDTA buffer was added to the DNA solution, and this was mixed with RNase A solution (final concentration of 20 μ g/ml) and incubated at 37 °C for 30 min. The DNA was extracted as above and precipitated with isopropylalcohol and sodium acetate. The purified DNA concentration was measured by absorbance at 260 nm.

Individual finger nail clippings were collected by the method described above from 7 healthy males and 5 healthy females on the medical staff, aged 25-55, and peripheral blood samples were also collected from 10 of them. Finger nail clippings were collected by the same method from 92 healthy male and 28 healthy female medical students in Kurume University School of Medicine, who were aged 21-40, and who also responded individually to the TAST.

Results

Extraction of genomic DNA and ALDH2 genotyping were successfully performed with 132 fingernail clippings including 10 manicured and smudgy nails. Figure 1B shows the DNA fragments extracted by the guanidium method from 3 manicured and 3 smudgy nails which had no DNA detectable by the proteinase K

method. The results of ALDH2 genotyping of the 10 samples completely matched the typings based on the corresponding peripheral blood.

Figure 2 shows that the processing of 2 to 3 heterozygous nails did not contaminate the subsequent ALDH2 genotyping of 4 atypical or mutant homozygotes (lanes 1-4), which have the 135 bp DNA fragment containing the ALDH2 exon 12, and of 2 typical or normal homozygotes (lanes 5-6) which have the 112 bp DNA fragment. The 23 bp DNA fragment is not visible on the electropherogram.

The mean recovery of DNA in the 10 nail samples was 0.33 μ g/10 mg nail.

The composition of the 3 genotypes, the 2 phenotypes and the genotype-phenotype agreement are shown in Table 1. The proportions of typical or normal homozygote, heterozygote, and atypical or mutant homozygote were 45% (41/92),

Fig. 2. A gel electropherogram showing no contamination by the previous processing of heterozygous nails for ALDH2 genotyping on 4 atypical or mutant homozygotes (lanes 1-4) and on 2 typical or normal homozygotes (lanes 5-6).

TABLE 1.
*Genotype-phenotype agreement of aldehyde dehydrogenase 2
 in 120 healthy Japanese*

Genotype	Phenotype observed by TAST*						
	Male		Female		Both Sexes		
	Non FL	FL**	Non FL	FL	Non FL	FL	Total
Normal homozygote	35	6	15	1	50	7	57
Heterozygote	12	34	1	5	13	39	52
Mutant homozygote	0	5	2	4	2	9	11
Total	47	45	18	10	65	55	120

*TAST : Tokyo-University ALDH2-Phenotype Screening Test

**FL : alcohol flusher

50% (46/92), and 5% (5/92), respectively for the males. Those for female were 57% (16/28), 21% (6/28), and 21% (6/28), and those for both sexes were 48% (57/120), 43% (52/120), and 9% (11/120), respectively. The sensitivity of the TAST for differentiating a normal homozygote from the other 2 genotypes based on the non-flushing reaction would be 85% (35/41) and the corresponding specificity would be 76% (39/51) for the males. Similarly, the values for females were 94% (15/16) and 75% (9/12), respectively, and the values for both sexes were 88% (50/57) and 76% (48/63), respectively.

Discussion

Polyacrylamide gel can be used instead of agarose for the electrophoresis, enabling the DNA fragment of 23bp to be visible on the electropherogram. However, it does not affect the ALDH2 genotyping.

The ALDH2 genotyping method described in this report is an improved method applicable not only to blood but

also to nail clippings, and it yields consistent genotyping results. It may be more flexible for mass processing in human genetics or in epidemiological studies than the use of blood samples.

The proportion of the 3 genotypes for all the subjects in the present study is comparable to that previously reported (Takase and Takada, 1991), but there seems to be a discrepancy based on sex, which is probably due to the small size of the data sample.

The TAST might be influenced by an individual's drinking experience, particularly for females, and it could affect the results of the TAST. However, the sensitivity or specificity of the TAST observed in the present study is comparable to that previously reported based on more than 500 workers (Takeshita et al. 1994). A relatively small sample size and neglect of the ADH genotype might partially explain the differences.

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