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Detection of Aldehyde Dehydrogenase 2 Gene Fragment Using Human Nail Clippings and Saliva

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An individual genotype of aldehyde dehydrogenase 2 (ALDH2) was observed when using nail clippings and saliva, respectively by the method consisting of DNA extraction, genomic DNA amplification by polymerase chain reaction, and gel electrophoresis of DNA fragment. Gene analysis using fingernail DNA was found to be effective in that they were easily to collect from human samples, in that they were kept storable at –80℃ until use, and in that DNA in the sample was very stable after repeated treatment of freezing and thawing.

Key words: ALDH (aldehyde dehydrogenase), genotype, phenotype, PCR (polymerase chain reaction), KOD (DNA polymerase derived from *Thermococcus kodakaraensios* KOD1 strain), homozygote, heterozygote

The oxidation of ingested ethanol to aldehyde is first catalyzed by alcohol dehydrogenase and then metabolized to acetic acid by six classes of aldehyde dehydrogenase (ALDH; EC.1.1.1.1) [1,2)]. ALDH2 has a low *Km* value and is regarded as the critical ALDH isoenzyme that determines an individual's intolerance to alcohol. Genotype-phenotype analysis of ALDH2 is essential for clinical, or epidemiological studies of alcohol-related diseases[3)]. According to the genotyping studies, the phenotypes relating to alcohol tolerance have been classified into three groups, consisting of normal homozygotes (NN), heterozygotes (NM), and mutant homozygotes (MM). A single G-to-A transition in the 3'-terminal sequences (size length:135 bp) of ALDH2 exon 12 region led to substitute glutamic acid for lysine in ALDH and resulted in the conversion of NN to NM or MM. The latter was intolerant to alcohol and highly prevalent among Japanese[4)]. Therefore, a rapid and reliable survey should be conducted among young female students that have not possessed alcohol-drinking habit, since it is not always feasible to collect data from a variety of female students.

The present study was undertaken to determine whether it was feasible or not to collect biological materials and to provide more information regarding the types of ALDH2 present in nails and in saliva.

Materials and methods

Collection of nails and saliva

After informed consents were obtained from 3 healthy Japanese female students that have no problems in alcohol ingestion, nails and saliva were collected, respectively. Nail clippings were rinsed once with ethanol and kept for 10 min at room temperature. Each specimen was cut into pieces (1.0 x 2.0 mm) , weighed and stored at -80°C until use. Saliva (0.2 – 0.5 ml) was collected from subjects after they had rinsed their mouth with deionized water and stored at -80°C. Alcohol patch tests and questionnaires

about drinking habit to the subjects were conducted simultaneously.

DNA extraction

Each specimen prepared from nail (1.0 – 20 mg) or saliva (10 – 20 μl) was transferred into microcentrifuge tube and incubated for 20 min at 55°C in 210 μl of urea - SDS solution by using a modification of the method of supplier (Isohair kit, Nippon gene Co.) and further incubated for 15 min at 55°C after adding 5 μl of proteinase K solution[5]. The mixture was then converted gently for 5 min with 200 μl phenol-chloroform-isoamylalcohol (25:24:1, v/v) at room temperature. After centrifugation at 14,000 rpm for 15 min at 4 °C, the upper layer was transferred to a new tube and mixed with 20 μl of 3M sodium acetate and 2 μl ethachinmate. After adding 400 μl of ethanol, the solution was kept at – 20°C for 30 min. The supernatant obtained by centrifugation was thoroughly decanted and the resulting precipitate was washed once with 70% ethanol and centrifuged. After drying in vacuum for 5 min, DNA was dissolved in 10 μl of TE buffer (pH 8.0).

PCR amplification

The allele-specific of ALDH2 gene was amplified with primer set of FR1, consisting of forward primer (F) (5'-CAAATTACAGGGTCAACTGCT-3') and reverse primer R1 (5'-CCACACTCACAGTTTTCTCTTC-3'), or set FR2, consisting of the forward primer (F) and reverse primer R2 (5'-CCACACTCACAGTTTTCTCT TT-3'). A total of 50 μl PCR solution, containing 1x PCR buffer, a template DNA, 0.5 mM $MgSO_4$, 0.2 mM dNTP, 0.4 mM forward primer, 0.4 mM reverse primer R1 or R2 and 1.25U KOD plus enzyme was pre-heated at 98 °C for 1 min ,and subjected to 35 cycles of amplification by PCR as follows: 98°C for 20 sec, 60°C for 20 sec, and 72°C for 45 sec. After the cycle, the mixture was once incubated for 5 min at 72°C and stored at 4°C.

Agarose gel electrophoresis

A 5- μl portion of the PCR product was analyzed in 3% agarose 21 gel[6]. Electrophoresis was carried out at 50 V for 30 min. Molecular markers employed was *Hae*III digest of ϕX174 DNA, consisting of 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118 and 72 base pairs. DNA was visualized after staining with ethidium bromide[7].

Results and discussion

Data from three healthy students were similar to the figures demonstrated in the typical patterns throughout the studies. Figure 1 and 2 clearly show that either 10 μl and more amount of saliva preparation or 5 mg and more of nail segments is sufficient enough to amplify a 135 bp DNA fragment containing ALDH2 exon 12 region (lanes 4,6, 8, and 10 in figure 1: lanes 4, 6,8, 10 and 12 in figure 2).

On the contrary, negative control tests conducted (lanes 2 and 3 in figures 1 and 2) indicated that the amplification procedures performed both in human saliva and in nails were not contaminated with other DNAs. Moreover, not any of 135 bp bands was seen when R2 was employed as the reverse primer (lanes 5, 7 ,9 and 11 in figure 1 and lanes 5, 7, 9, 11 and 13 in figure 2).

Faint bands of staining located at around 50 bp were visible in lanes 3 thorough 10 indicated the proper proceedings of the PCR (positive control).

As demonstrated in figures 3 and 4, 135 bp DNA fragment containing ALDH2 exon12 was not destructed even after 20 cycles of freezing and thawing.

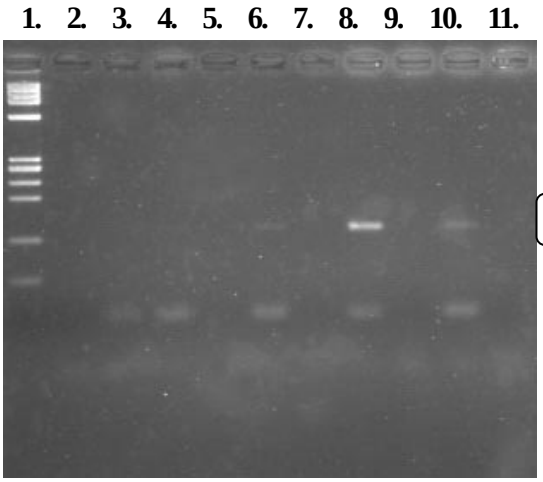

Fig. 1. Electrophoresis pattern of the PCR product of a 135 bp DNA fragment containing ALDH2 exon 12 from human saliva.

Lane 1, ϕX174/ *Hae*III; lanes 2 and 3, denoted the respective template DNA was omitted (negative control). Either 1-μl, 5-μl, 10-μl or 20-μl portion of saliva solution was employed in PCR mixture, and 5-μl of the product was applied to lanes 4 and 5, 6 and 7, 8 and 9, and 10 and 11, respectively. R2 was used for reverse primer in lanes 2, 4, 6, 8 and 10., in figure 1, and lanes 3,5,7,9 and 11in figure 2.

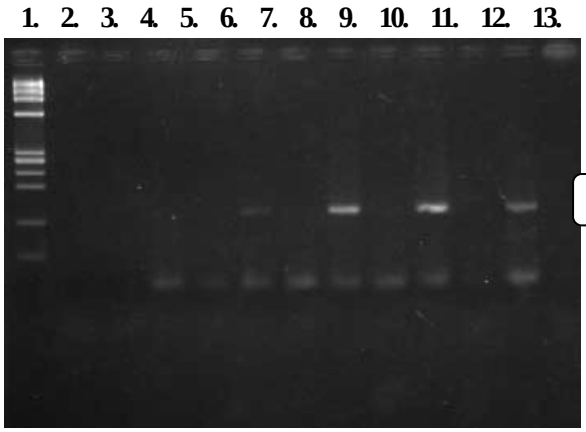

Fig. 2. Dependency of nails clippings extract concentration on PCR production of a 135 bp DNA fragment containing ALDH2 exon 12

Lanes 4 and 5 representsDNA extract from 1 mg of nail clippings. Lanes 6 and 7, 8 and 9, 10 and 11, and 12 and 13 demonstrate the DNA extracts from 2 mg, 5 mg, 10 mg and 20 mg nail clippings, respectively. The other experimental procedures and denotes in the figure were the same as described in figure 1.

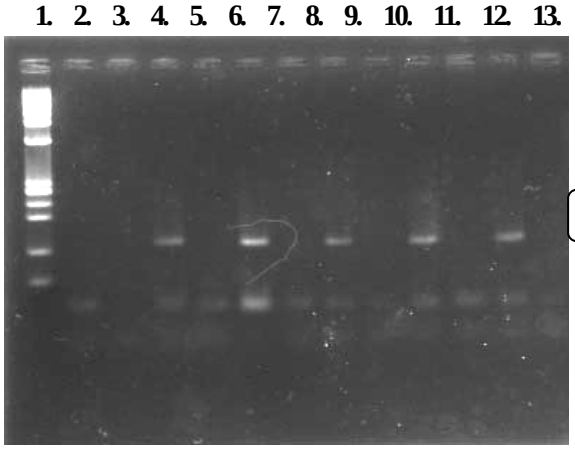

Fig. 3. Effect of repeated freezing and thawing on PCR production of a 135 bp DNA fragment containing ALDH2 exon 12 in saliva.

Lanes 4 and 5 represents 1 cycle of freezing and thawing treatment of the template DNA. Lanes 6 and 7, 8 and 9, 10 and 11, and 12 and 13 demonstrated the repeated times of 5, 10, 15, and 20,respectively of freezing and thawing. The other experimental procedures and denotes in the figure were the same as described in figure 1.

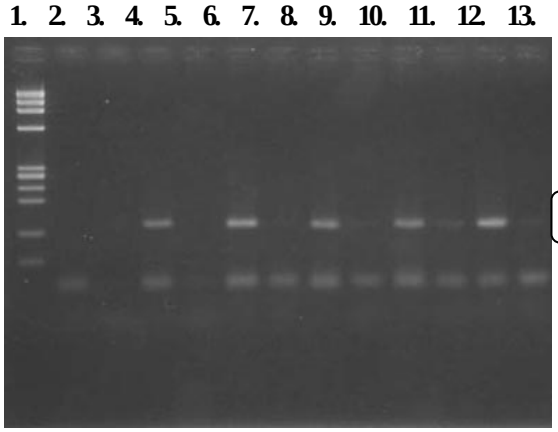

Fig. 4. Effect of repeated freezing and thawing on PCR production of of a 135 bp DNA fragment containing ALDH2 exon 12 in nail clippings.

Lanes 4 and 5 represent 1 cycle of freezing and thawing. Lanes 6 and 7, 8 and 9, 10 and 11, and 12 and 13 demonstrate the repeated cycles of 5, 10, 15, and 20,respectively. The other experimental procedures and denotes in the figure were the same as described in figure 1.

From these results together with the alcohol patch tests and questionnaires, the genotype of subject was normal homozygotes (NN), and not heterozygotes (NM) nor mutant homozygotes (MM).

Nail clippings are relatively easy to obtain from human volunteers especially when they are restricted to female students. Plucking hair roots with attached bulbs from the people's scalp was not successful, since it caused sometimes pains on the volunteers and template DNA was extremely low in hair sample preparations (data not shown).

Genetic and epidemiological studies on ALDH2 using nails from more than 150 students are now in progress.

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