Single-Strand Conformation Polymorphism Analysis for Alcohol Dehydrogenase 2 (ADH2) Genotyping Using Nail Clippings, Atushi Nishiyori, Ritsu Sakata, and Katsuhiro Fukuda (Department of Public Health, Kurume University School of Medicine, 67 Asahi-Machi, Kurume 830, Japan; * author for correspondence: fax 81-942-31-7698, e-mail sakata@mail.med.kurume-u.ac.jp)

The oxidation step in the metabolism of ethanol to acetaldehyde is catalyzed by six classes of isoenzymes of alcohol dehydrogenase (ADH; EC 1.1.1.1) (1). The ADH1, ADH2, and ADH3 genes encode the class I enzymes α, β, and γ, respectively. The class I enzymes play an important role in the metabolism of ingested ethanol and form homo- and heterodimeric isoenzymes. The largest kinetic differences among the three polymorphic variants were found in the β class of enzymes: β1, encoded by ADH21; β2, encoded by ADH22; and β3, encoded by ADH23. We have focused on ADH21 and ADH22 because β2 is highly prevalent among Orientals (2) and β3 has been identified in only Africans (3). A G-to-A transition leads to conversion of arginine-47 (β1) to histidine (β2) (4). The atypical enzymes involved in the β2 variant, encoded by ADH22 subunit(s), have high activities, as indicated by the Vmax values (5), but the significance of ADH2 genotypes in alcohol-related problems is still controversial (6–10). We have previously reported the following: (a) nucleic acids extracted from nail clippings may be a suitable material for DNA analysis by PCR in a population study; (b) specific amplification of the ADH2 gene was carried out without simultaneous amplification of ADH1 and ADH3; and (c) ADH2 genotyping in fingernails can be achieved by PCR coupled with restriction fragment length polymorphism (PCR-RFLP) analysis (11). The standard PCR-RFLP method applied to a large number of nail samples, however, yielded occasional questionable electropherograms. A few samples, classified as ADH22/ADH22, showed a faint band with a mobility similar to that of the digested band of ADH22, and another sample, classified as ADH22/ADH22, showed a very thin undigested band of ADH21. These results suggest incomplete digestion. Here we report an alternative method of ADH2 genotyping, based on single-strand conformation polymorphism (SSCP), that is rapid, inexpensive, and reliable.

After informed consent was obtained from 119 healthy Japanese students, nails were collected as described previously, and nucleic acids were extracted from the nail parings by guanidine thiocyanate (11). The ADH2 allele was amplified as reported previously (11). The ADH2-specific allele was amplified with primer set 1 [M3FCG (5′-CTGTAGGAATCTGTGCA-3′) and M3RT (5′-CCTCTCACAACACTCTC-3′)] and the ADH2-specific allele with primer set 2 [M3FCA (5′-CTGTAGGAATCTGTGCA-3′) and M3RT] under previously reported conditions except for an annealing temperature of 62 °C. RFLP analysis was carried out as described previously (11).

A 2-μL portion of the PCR product was diluted with 4 μL of the SSCP buffer (475 mL/L formamide, 0.5 g/L xylene cyanol, 0.2 g/L bromphenol blue, 5 mmol/L Tris, and 0.5 mmol/L EDTA). The samples were heated to 95 °C for 5 min, immediately cooled in ice-water, and electrophoresed in a Gene Gel Excel 12.5/24 (Amersham Pharmacia Biotech). Electrophoresis was carried out at 600 V (25 mA) for 80 min at 5 °C. DNA fragments were revealed by silver staining with the Plus One DNA silver staining reagent set (Amersham Pharmacia Biotech).

When the results of SSCP analysis and standard RFLP analysis were not consistent, those samples were reanalyzed by both SSCP analysis and a revised RFLP analysis described below. Nucleic acids were again extracted from the nail clippings. After the DNA was purified (11), it was washed in 100 μL of 700 mL/L ethanol and dissolved in 20 μL of Tris-EDTA buffer. After amplification of the ADH2 allele, 2 μL of the PCR products was used for the SSCP analysis, and 20 μL of the PCR products was precipitated with ethanol. The centrifuged pellets were then washed with 700 mL/L ethanol. After centrifugation, the pellets were dried and dissolved in 18 μL of Tris-EDTA buffer; 6 μL of this DNA solution was digested overnight in a 10-fold volume of MslI at 37 °C, in contrast to the method for the standard RFLP analysis (11). The digests underwent electrophoresis in an 8% polyacrylamide gel and were then visualized by ethidium bromide staining.

The nucleic acid sequences of the PCR products amplified with primer sets 2 and 3 were analyzed by a direct sequencing technique. The sequences of the ADH21 and ADH22 allele-specific PCR products amplified with the ADH21 and ADH22 allele-specific primers were confirmed by direct sequencing to be essentially consistent with the reported sequences of ADH21 and ADH22 (12), respectively.

The ADH21-specific PCR product produced the bands shown in lane 2 of Fig. 1B, whereas the ADH22-specific PCR product produced the bands shown in lane 4. The latter are clearly different from ADH21. The mobilities of the PCR products from three ADH2 genotypes (ADH21/ADH21, ADH21/ADH22, and ADH22/ADH22), which had been previously determined by PCR-RFLP analysis, were identical to those of the allele-specific PCR products, and the bands from each of these three genotypes were clearly distinguished from each other (Fig. 1C). The results of the standard RFLP analysis and those of the SSCP analysis were consistent in 115 of 119 samples, but not in 4 samples. ADH2 genotyping in three of those four samples showed ADH21/ADH21 by standard RFLP analysis but ADH21/ADH22 by SSCP analysis, and one of the four samples showed ADH21/ADH22 by standard RFLP analysis but ADH21/ADH22 by SSCP analysis. However, the results of the revised RFLP analysis applied to the four samples were completely consistent with those of the SSCP analysis.

These results demonstrate that ADH2 genotyping can be carried out successfully by SSCP analysis. We have already shown that nucleic acids can be extracted from nail clippings (11). The differences between the results of genotyping by standard RFLP analysis and those of genotyping using the revised RFLP and SSCP analyses are
not likely to be attributable to contamination of the DNA because all of the results obtained by the revised RFLP analysis of DNA solutions in which the standard RFLP analysis produced questionable electropherograms completely coincided with the SSCP analysis of DNA extracted again from the same finger nails. The genotyping results obtained for ADH2*1/ADH2*2 by SSCP analysis may be mistaken for those obtained for ADH2*1/ADH2*1 by standard RFLP analysis. In addition, ADH2*2/ADH2*2 results obtained by SSCP analysis may be mistaken for ADH2*1/ADH2*2 results obtained by standard RFLP analysis because of incomplete digestion of PCR products by the restriction enzyme, MsiI, because of contamination by various substances (e.g., guanidinium thiocyanate), which may inhibit the activity of MsiI. Nucleic acids were successfully extracted from some dirty nails or manicured nails with the present guanidinium thiocyanate method, but not with the proteinase K method; therefore, nails may be a suitable material for mass processing in epidemiologic studies (13). SSCP analysis, because it does not use restriction enzymes, may be preferable to RFLP analysis in genomic DNA analysis using nails.

References


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Increased porphobilinogen (PBG) in urine is pathognomonic of an attack or “crisis” of acute porphyria (acute intermittent porphyria, variegate porphyria, hereditary coproporphyria); the absence of increased urinary PBG in urine for excess PBG and its derivatives excludes the diagnosis. Some patients, especially with acute intermittent porphyria, excrete excess PBG even in remission, but in an attack, this increases above their generally characteristic “basal” concentrations (1). Thus, “screening” of urine for excess PBG and its measurement have an essential role in the initial diagnosis and management of such patients and in their continuing care.