Brief Communication

Population Genetic Studies on Aldehyde Dehydrogenase Isozyme Deficiency and Alcohol Sensitivity

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SUMMARY

Population genetic studies on aldehyde dehydrogenase polymorphism using hair-root samples were performed on Europeans, Libyans, Sudanese, Egyptians, Kenyans, Vietnamese, Japanese, Indonesians, Chinese, Thais, and South American Indians. A possible correlation between ALDH I deficiency and sensitivity to alcohol in Oriental populations is discussed.

INTRODUCTION

The NAD-dependent aldehyde dehydrogenase (ALDH, E.C.1.2.1.3) catalyzes the oxidation of various aldehydes in humans. Greenfield and Pietruszko [1] found two isozymes of ALDH, a faster-migrating isozyme with a low $K_m$ for acetaldehyde and a slower-migrating one with a high $K_m$ for acetaldehyde in human liver.

The existence of two additional isozymes of ALDH with a high $K_m$ for acetaldehyde in various organs and tissues was reported by us [2, 3]. The four isozymes are termed ALDH I, II, III, and IV according to their decreasing electrophoretic migration and increasing isoelectric point.

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In our previous studies on 90 autopsy liver extracts from Germans, we found no differences in the isozyme pattern of ALDH. However, in about half of 40 Japanese autopsy samples, the fast-migrating ALDH I isozyme was missing [4, 5]. Subsequently, Teng reported similar findings in Chinese subjects but found only about a 4% deficiency in Asiatic Indians [6].

Population genetic studies on alcohol metabolizing enzymes in human biopsy livers are not practicable. By developing sensitive micromethods, we could detect enzyme activity and isozyme bands in skin extracts and cultured skin fibroblasts [2] as well as in hair roots [7]. Here we report the incidence of the deficiency of ALDH I in various populations.

SUBJECTS AND METHODS

Two hundred twenty-four Europeans, 15 Kenyans, 169 Liberians, 160 Sudanese and Egyptians, 110 Thais, 196 Chinese, 35 Indonesians, 184 Japanese, 82 Vietnamese, and 33 Indians from Ecuador were analyzed. The subjects, both male and female, were between 15 and 60 years old.

Collection and Transport of Human Hair Roots

About 40 hair roots were required as the minimum quantity from each subject. Hairs with visible bulb and sheath (hair roots) were plucked preferably from the middle of the head and placed into small plastic vials (e.g., Eppendorf microtubes) with the roots facing the bottom of the container. The other ends of the hairs remained outside the tube, which was capped tightly. The hair samples were transported in dry ice by airmail so as to reach our institute within 4–6 days after plucking.

Separation and Detection of ALDH Isozymes

Hair root lysate preparation: 30–50 hair roots (with visible bulb and sheath, not more than 6 days old) were lysed in 100 μl distilled water by repeated freezing and thawing (three cycles) in a small plastic vial. The lysate was thoroughly mixed using a whirl mix and was used for isoelectric focusing (IEF).

Gel composition: Size—18 × 12.5 × 0.1 cm; 7 ml 29.1% acrylamide (w/v), 7 ml 0.9% bisacrylamide (w/v), 14 ml 15% sucrose; ampholyte solution—1 ml of pH 3.5–10.0

FIG. 1.—ALDH isozyme patterns obtained by IEF of liver extract (1) and hair root lysates (2–5); 2 corresponds to a Thai subject deficient in ALDH I isozyme.
and 1 ml of pH 4-6, 15 mg ammonium persulfate. The gel was polymerized for 1 hr at room temperature.

**Electrodes:** 2.50-mm thick paper strips soaked with 0.3% H₃PO₄ and 0.5% NaOH for anode and cathode ends, respectively, were put on two sides of the gel. Electrodes were put on these strips.

**Sample application:** 50 µl lysate was applied on 0.4 × 3.0 cm Whatman no. 1 paper strips at the cathodic side of the gel.

**Focusing:** Focusing was done using a constant voltage of 1,000 V with a maximum of 45 mA for about 150 min.

**Staining:** Enzyme activity staining was used.

**Solution A:** 10 mg MTT, 60 mg NAD⁺, 50 mg pyruvate, 0.5 mg Meldola blue (Boehringer, Mannheim, West Germany) were dissolved in 150 µl H₂O and mixed with 225 µl 98% propionaldehyde in 3 ml of 0.1 M pyrophosphate buffer, pH 8.0, containing 0.01 M diethanolamine.

**Solution B:** 250 mg agarose was dissolved by heating in 22 ml pyrophosphate buffer of the same composition as above and maintained at 50°C.

Solutions A and B were mixed just before use, and the mixture was overlayed on the polyacrylamide gel after completion of the IEF. The gel surface was then covered with a plastic foil and left for about 2 hrs at 37°C in an incubator in the dark. Dark blue-violet formazan zones or bands due to ALDH activity were visible in the anodic region (isoelectric points between 4 and 6) against a yellow background. The agar-overlayer is removed for a better assessment of the bands.

RESPECTS AND DISCUSSION

A comparison of ALDH isozyme patterns from liver extracts and hair-root lysates after IEF is demonstrated in figure 1. Normal and deficient types of ALDH are clearly distinguishable.

The frequency of ALDH I deficiency in different populations is shown in table 1. The deficient type could not be detected in Europeans, Egyptians, Sudanese, Liberians, or Kenyans. However, 35%-57% of the Oriental populations showed ALDH I deficiency. In Thais from the northern part of Thailand, only 8% of the subjects were deficient for ALDH I. In Highland Indians from Ecuador, 69% were deficient for ALDH I.

Initial flushing and other vasomotor symptoms after alcohol ingestion, which is observed frequently in Orientals, seems to correlate with ALDH deficiency.

<table>
<thead>
<tr>
<th>Population</th>
<th>No.</th>
<th>% Deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indians (Ecuador-Highland)</td>
<td>33</td>
<td>69</td>
</tr>
<tr>
<td>Vietnamese</td>
<td>82</td>
<td>57</td>
</tr>
<tr>
<td>Japanese</td>
<td>184</td>
<td>44</td>
</tr>
<tr>
<td>Indonesians</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Chinese</td>
<td>196</td>
<td>35</td>
</tr>
<tr>
<td>Thais (Northern Thailand)</td>
<td>110</td>
<td>8</td>
</tr>
<tr>
<td>Egyptians and Sudanese</td>
<td>160</td>
<td>0</td>
</tr>
<tr>
<td>Liberians</td>
<td>169</td>
<td>0</td>
</tr>
<tr>
<td>Kenyans</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Europeans</td>
<td>224</td>
<td>0</td>
</tr>
</tbody>
</table>
Such individuals are supposed to have an inability to metabolize acetaldehyde effectively in the absence of ALDH I isozyme [4]. We found ALDH I deficiency in the hair roots of flushing subjects only [7, 8]. The flushing subjects also showed an elevated blood acetaldehyde level [9].

The population genetic data presented here further confirm the wide prevalence of ALDH deficiency among Oriental populations. The initial sensitivity to alcohol may exert an aversive effect toward their alcohol consumption and, consequently, on the incidence of alcohol-related problems. Epidemiological data support this notion, as the rate of alcoholism in Japanese, for example, is significantly less than in the Europeans and Northern Americans.

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REFERENCES

1. GREENFIELD NJ, PIETRUSZKO R: Two aldehyde dehydrogenases from human liver. Isolation via affinity chromatography and characterization of the isozymes. Biochim Biophys Acta 483:35–45, 1977