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Pharmacogenetics of Alcohol Sensitivity

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GOEDDE, H. W., D. P. AGARWAL AND S. HARADA. *Pharmacogenetics of alcohol sensitivity*. PHARMACOL BIOCHEM BEHAV **18**: Suppl.1, 161–166, 1983.—The metabolism of acetaldehyde has received considerable attention in the past few years due to its toxic effects and possible importance in pharmacogenetics. Recent studies have demonstrated rapid progress concerning the multiple molecular forms of ADH and ALDH and their genetic variants. The isozymes of ALDH may play an important role in the biological sensitivity to alcohol in certain ethnic groups and also in the pathogenesis of alcohol related organ damage. A protective effect of ALDH I deficiency against alcoholism seems to exist in Japanese.

Isozyme deficiency Pharmacogenetics Alcoholism Aldehyde dehydrogenase Alcohol sensitivity Facial flushing Acetaldehyde

MANY of the so-called drug metabolizing enzymes are detectable in multiple molecular forms. Alcohol is a drug, an environmental substance or xenobiotic which is often a potentially toxic chemical which may cause damage to certain individuals, but not to others. In this concern the study of genetic aspects of ecology is of great importance [8].

Alcohol metabolizing enzymes have been investigated by us in autopsy and biopsy tissue specimens, cultured fibroblasts, erythrocytes, plasma, and hair roots. The present paper deals with the heterogeneity of the enzymes alcohol dehydrogenase and aldehyde dehydrogenase, using different techniques: starch gel electrophoresis, isoelectric focusing, Km determination, substrate specificity, inhibitor sensitivity, etc.

Various social, environmental and genetic influences have been found to be involved in individual variations of alcohol intoxication effects. Subjective symptoms of alcohol sensitivity are much more frequent in Orientals than in Caucasians. Facial flush response has been noted in 50 to 80% in these populations, and only in about 10% Caucasians (Table 1).

Different explanations have been suggested regarding higher blood acetaldehyde levels frequently observed in Japanese and Chinese individuals after alcohol drinking such as faster absorption rate of ethanol, differential base rates of alcohol metabolism, differences in the clearance rate of acetaldehyde among different racial groups and the genetic polymorphisms of alcohol dehydrogenase or aldehyde dehydrogenase.

Acetaldehyde was given exogenously first by Asmussen in 1948 [3] showing its intoxication effect. It was suggested that acetaldehyde causes the release of norepinephrine.

The main enzyme responsible for alcohol degradation is alcohol dehydrogenase. The separation of alcohol dehydrogenase (ADH, EC 1.1.1.1) isozymes are shown in Fig. 1. A variant form with 6 times higher activity was termed atypical ADH by von Wartburg [26] because this form is rare in Europe. However, it is very frequent in Japan and differs from typical ADH in pH-optimum, substrate specifity and inhibition by thiouria (Table 2). Until recently it was believed that the atypical ADH may be responsible for the adverse reactions to alcohol [23,25]. However, no significant difference in the rate of alcohol degradation between carriers of normal and atypical ADH has been observed [5,21]. Also Mizoi [18] found no difference concerning elimination rates of alcohol between individuals with and without flushing reactions.

The second major enzyme in alcohol metabolism, aldehyde dehydrogenase (ALDH EC 1.2.1.3) may possibly be the determining factor in alcohol sensitivity. Two isozymes of ALDH have been reported and their molecular weight, subunit composition and coenzyme requirement were studied [13,27]. We could show that human liver ALDH consists of at least four main isozymes with different electrophoretic migrations [12,14]. The distribution of these isozymes in different tissues is shown in Fig. 2. The fast migrating ALDH I and II (corresponding to E_2 and E_1 isozymes) of Greenfield and Pietruszko [13] as well as ALDH IV were mainly detected in liver and kidney; ALDH III was observed in stomach, lung and scalp [9].

We could demonstrate these isozymes also in skin biopsies, cultured fibroblasts, lymphocytes, erythrocytes, and hair roots [9, 11, 12]. While in fibroblasts, scalp skin, and hair roots mainly ALDH I and II were detected, erythrocytes showed only the ALDH II band. In serum and platelet extracts no ALDH isozymes could be detected. The different isozymes were purified by column isoelectric focusing and other procedures from liver and stomach and the Km values for aldehyde and NAD^+ were estimated (Table 3).

In 90 German autopsy liver specimens we always found the same ALDH isozyme pattern; however, in half of about 40 Japanese livers, the ALDH I isozyme was missing [10,15]. A comparison of ALDH isozyme pattern is shown in Fig. 3. Samples 1, 3, and 4 show the deficient or unusual ALDH type, 2 and 5 show both the isozymes ALDH I and II (the usual type). The pH optimum for the usual and deficient liver

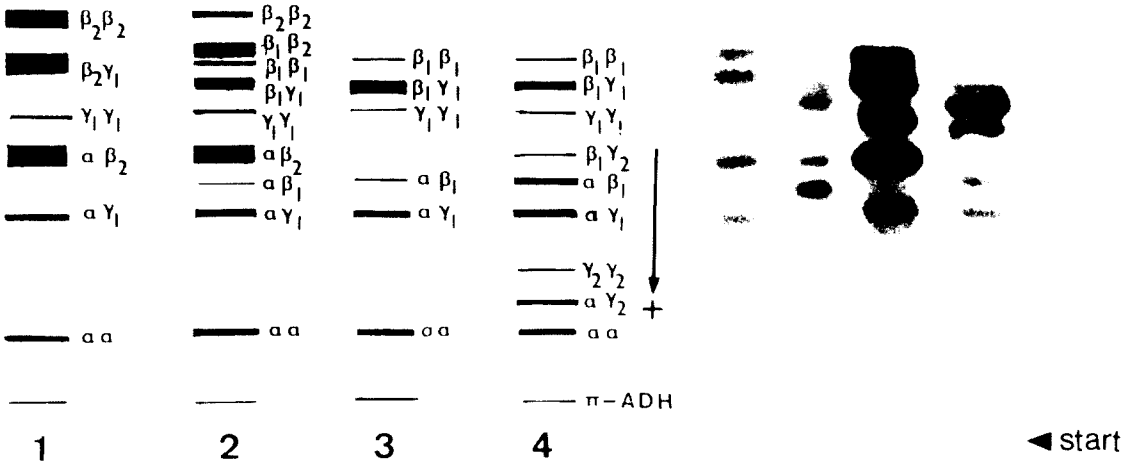

FIG. 1. Liver ADH isozyme patterns obtained by prolonged high voltage starch gel electrophoresis. 1, $ADH_2$2, $ADH_3$1;, 2, $ADH_2$2-1, $ADH_3$1; $ADH_2$1, $ADH_3$1; 4, $ADH_2$1, $ADH_3$2-1. (Reprinted from Harada *et al.* [15] with permission.)

TABLE 1

ALCOHOL SENSITIVITY (FACIAL FLUSHING) IN DIFFERENT POPULATIONS AND ETHNIC GROUPS

Subject Group	Frequency of Sensitivity %	Reference
Caucasians		
Europeans	4	[29]
Europeans	10	[31]
North Americans	12	[6]
American Indians		
North American Indians	50	[30]
North American Mongoloids	80	[30]
Americans with Mongoloid and European Ancestory	90	[30]
Orientals		
Japanese	58	[18]
Japanese	85	[19]
Japanese, Korean, Chinese, Taiwanese	83	[29]
Chinese	57	[31]
Hapa Haole (Hawaii)	60	[28]
Vietnamese	60	[11]

TABLE 2

FREQUENCY OF HUMAN LIVER ATYPICAL ALCOHOL DEHYDROGENASE IN DIFFERENT POPULATIONS

Subject Group	% Atypical	Reference
Swiss	20	[26a]
English	5	[5]
English	10	[22]
German	8.7	[17]
German	9	[14]
German	14	[21]
Japanese	88	[7]
Japanese	98	[20]
Japanese	85	[23]
Chinese	85	[24]
Indians	0	[24]
Vietnamese	86	[11]

TABLE 3

K_m VALUES OF 4 ISOZYMES FOR PROPIONALDEHYDE AND NAD AT pH 9.5 (LINEWEAVER-BURK PLOTS)

Isozymes	K_m values aldehyde	(μM) NAD
ALDH I	3.5	65
ALDH II	94	24
ALDH III	930	28
ALDH IV	1,400	49

ALDH was 9.5 and 9.0, respectively; inhibition by disulfiram was about 30% and 80%, respectively. Similar findings in Chinese autopsy liver samples have confirmed our studies [24a].

In view of the polymorphic nature of ALDH in Japanese we postulated that the frequently observed alcohol sensitivity in Japanese could be due to the inability of these individuals to metabolize acetaldehyde quickly in the absence of ALDH I [10]. These individuals consequently would be exposed to elevated blood acetaldehyde concentrations for longer periods. The detection of ALDH isozymes in human biopsies and cultured fibroblasts opened the possibility of studying the interindividual ALDH variation in human subjects [9]; however, due to lack of a suitable source of ALDH activity, it is difficult to get large numbers of biopsy samples or skin punches. Also, cell culturing is very time consuming.

Using sensitive isoelectric focusing techniques, ALDH can also be demonstrated in hair roots [11]. A comparison of

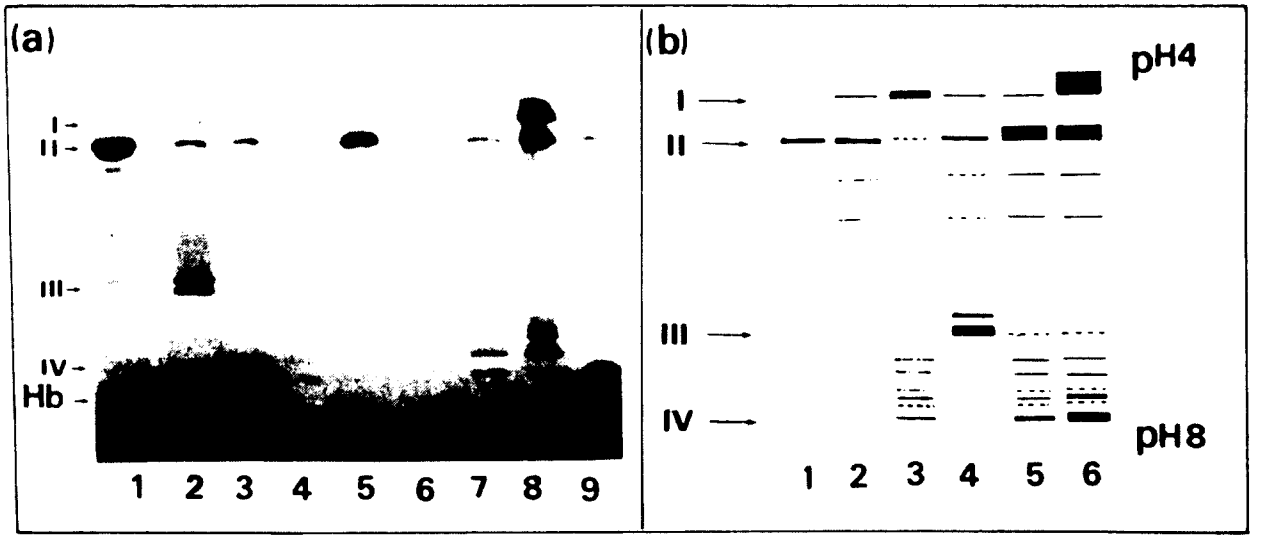

FIG. 2. Isoelectric focusing of ALDH isozymes. (a) 1: stomach, 2: lung, 3: muscle, 4: heart, 5: intestine, 6: skin, 7: kidney, 8: liver, 9: erythrocytes. (b) diagrammatic scheme of isozyme pattern in different tissues: 1: erythrocytes, 2: intestine, muscle, spleen, brain, 3: heart, 4: lung, stomach, skin, b: kidney, 6: liver. Main isozyme sets are indicated by I, II, III and IV. (Reprinted from Goedde *et al.* [9] with permission.)

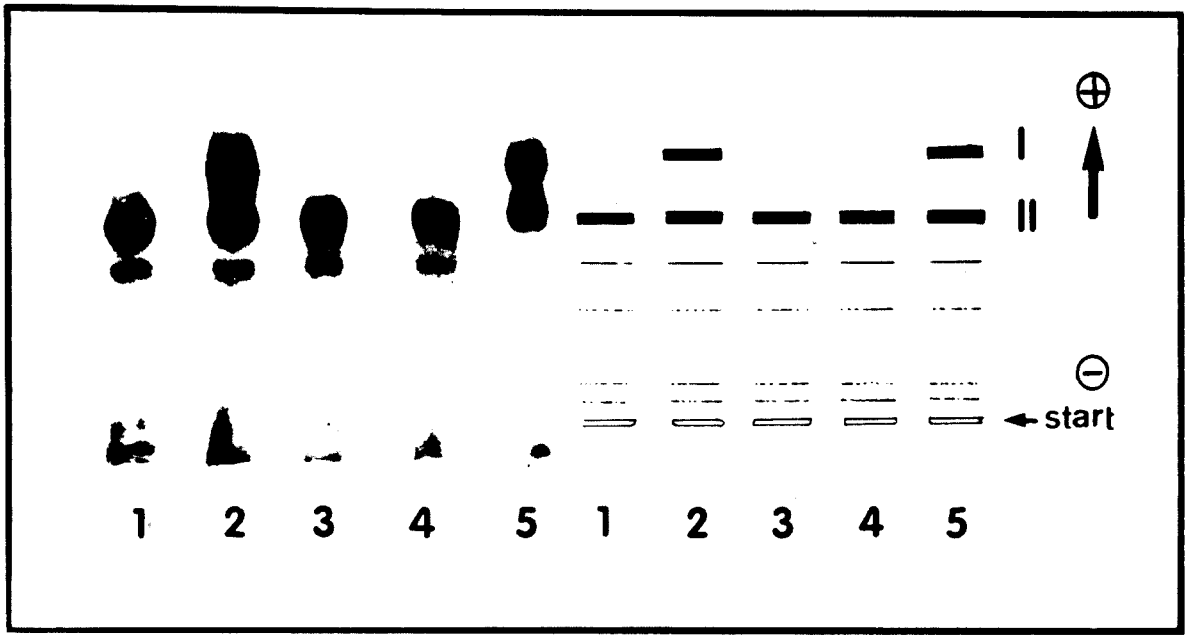

FIG. 3. Liver ALDH isozyme patterns obtained by starch gel electrophoresis in Japanese (1–4) and European liver samples (5). 1, 3 and 4 correspond to unusual type; 2 and 5 correspond to usual type. (Reprinted from Goedde *et al.* [10] with permission.)

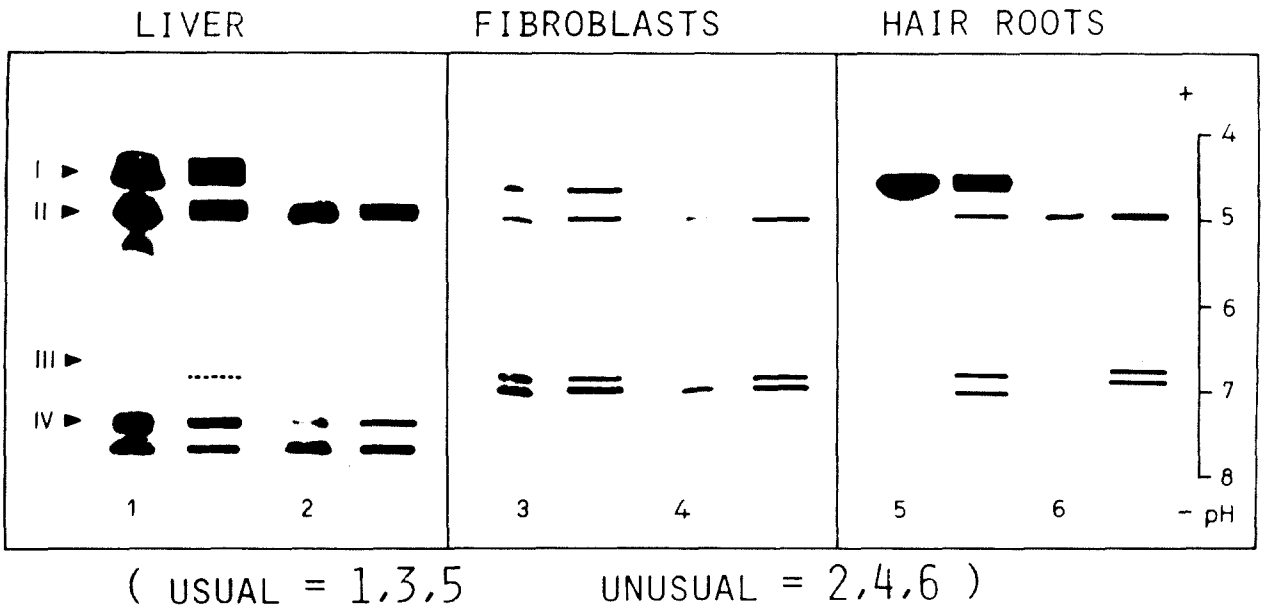

FIG. 4. Isoelectric focusing pattern of normal and deficient ALDH isozymes: liver (1,2), fibroblasts (3,4), hair roots (5,6). I-IV denote main isozymes. (Reprinted from Goedde *et al.* [11] with permission.)

TABLE 4

KINETIC PROPERTIES OF ISOLATED ALDH-ISOZYMES I AND II FROM HUMAN LIVER AND SCALP

	ALDH I	ALDH II	ALDH I	ALDH II
	Liver		Scalp	
K_m for acetaldehyde	3 μM	32 μM	4 μM	48 μM
K_m for NAD^+	70 μM	8 μM	200 μM	20 μM
pH-optimum	9.0	9.0	9.0	9.0
Isoelectric point (pI)	4.95	5.30	4.95	5.30
Inhibition by Disulfiram (20 μM)	44%	68%	46%	47%
Thermostability 50°C, 120 min	unstable	stable	unstable	stable

the usual and deficient ALDH types with those from liver and fibroblast lysates are shown in Fig. 4.

Mixtures of the purified ALDH isozymes from liver, hair roots, and skin revealed similar properties in electrophoresis. Isozymes from liver and scalp possess quite similar kinetic properties as shown in Table 4.

While in Europeans, Egyptians, Sudanese, and Liberians, no ALDH I deficiency was observed, various Oriental populations (Japanese, Chinese, Vietnamese, Thais, and American Indians) investigated by us showed ALDH deficiency with different frequencies [12].

Studies in European and Oriental families showed that ALDH deficiency may be transmitted autosomally (Fig. 5).

Genetic deficiency of ALDH I seems to be the determining factor in alcohol sensitivity: higher acetaldehyde concentrations in flushing subjects observed by many workers represents only a characteristic state while the genetic polymorphism of ALDH represents a genetic trait [1, 10, 15].

A correlation between facial flushing and elevated blood acetaldehyde levels in conjunction with ALDH deficiency was established by studying healthy Japanese individuals with normal or deficient ALDH I in the hair roots. Blood alcohol and acetaldehyde levels were estimated after alcohol loading by gas chromatography [16]. Flushing and cardiovascular symptoms were experienced by all those subjects with the deficient type. While the ethanol levels (10.5 mM) were almost the same in both usual and deficient groups, acetaldehyde concentrations were significantly higher in the ALDH I deficient group (35.5 μM) in comparison to about 2.0 μM in the normal group [16].

These data support our hypothesis [10] that individuals deficient for ALDH I may be at higher risk to acetaldehyde related organ damage or to complications in utero resulting from maternal alcohol abuse.

As about 40–50% of individuals in Japan have this deficient type of ALDH, these subjects may have a possible

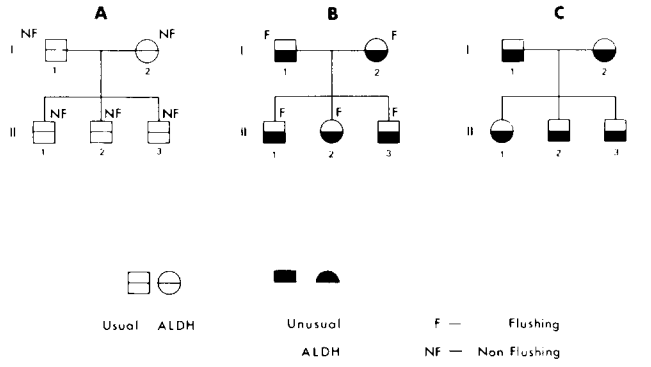

FIG. 5. Pedigrees of German (A), Japanese (B) and Vietnamese (C) families.

protection against alcoholism because of a physiological aversion against alcohol drinking. Indeed, the incidence of alcoholism in Japan is considerable lower than in other Western counttries.

The metabolism of biogenic amines and neurotransmitters in connection with aldehyde dehydrogenase deficiency seems to be of high interest. It is not known whether the biological hypersensitivity prevents euphoric effects of alcohol at a later stage of intoxication. It is to be expected that individuals with increased acetaldehyde levels would experience numerous toxic effects. An important question is whether elevated acetaldehyde could explain a propensity for alcoholism by the production of higher levels of catecholamine-condensation products. Tetrahydropapaveroline (THP), which is formed by reaction of dopamine and 3,4-dihydroxyphenyl acetaldehyde (DOPAL), may play an important role in addictive effects of ethanol [4]. DOPAL is produced in various mammalian tissues via oxidative deamination of dopamine, serotonin, and norepinephrine.

Preliminary investigations in different brain regions after subcellular fractionation of autopsy samples showed that the activity with DOPAL as a substrate was found to be localized mainly in the mitochondrial, microsomal and cytosolic fractions of corpus striatum. The characteristics of this ALDH were similar to those obtained with acetaldehyde as a substrate [2].

More recently studies (A. Yoshida, Personal communication) have shown that Japanese livers deficient in the ALDH I form contains enzymatically inactive but immunologically cross reactive material. These findings suggest that the absence of ALDH I activity may be due to a structural mutation and not a regulation mutation, gene deletion or nonsense mutation. Therefore, the genetic variation in ADH and ALDH isozymes may be responsible for susceptibility to alcohol-related behavioral complications and at the same time may exert protective effects against alcoholism in some populations.

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