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MITOCHONDRIAL MALIC ENZYME POLYMORPHISM IN JAPANESE POPULATION

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Summary Mitochondrial malic enzyme (ME_M) was examined by starch gel electrophoresis in 126 liver samples and 43 leukocytes from adult subjects in Japanese. The estimated gene frequencies of ME_M were 0.56 for ME_M^1 and 0.44 for ME_M^2 respectively, in this population. There were differences in the gene frequencies between the Japanese population and the population of the other races. These findings show that the gene frequency of $ME_M^1=0.56$ of Japanese is lower than that of North Americans (0.67), American Negroes (0.83), European Caucasians (0.67) and Southwestern Germans (0.67) reported until now.

This investigation coincided with the gene frequencies of the Indian population. From the results, it was found that the mitochondrial malic enzyme of leukocytes could be applicable in the field of forensic medicine.

INTRODUCTION

Malate dehydrogenase [EC 1.1.1.37] MDH and malic enzyme [EC 1.1.1.40] ME have been shown to coexist in two forms, cytoplasmic and mitochondrial. MDH catalyzes the following reversible reaction in the citric acid cycle. L-Malate + NAD $\rightleftarrows$ oxaloacetate + NADH. ME reversibly catalyzes the oxdative decarboxylation of malate and is a link between the glycolytic pathway and the citric acid cycle. L-Malate + $NADP^+ \xleftarrow{Mg^{2+}Mn^{2+}}$ pyruvate + CO_2 + H^+ + NADPH. Cohon (1971) described genetic polymorphism of human mitochondrial malic enzyme and designated MOD-2A, MOD-2AB, MOB-2B as three common patterns of malate oxidative decarboxylase. Burchell described that ME_M is present in appreciable quantities in human heart, brain, adrenal cortex and kidney, but is absent from blood, adipose tissue and skeletal muscle (Burchell, 1977). However, Siebert *et al.* (1979) confirmed that ME_M variation existed in human leukocytes by using 10% Triton X-100 solution and Tris-histidine buffer at pH 7.6. The polymorphism of ME_M has been reported in North Americans and American Negroes (Cohen and Omenn, 1972), European Caucasians (Povey *et al.*, 1975; Burchell *et al.*, 1977; Saha

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et al., 1978), Southwestern Germans (Siebert *et al.*, 1979) and East Indians (Ghosh *et al.*, 1980).

In this report we present the distribution of the gene frequencies of ME_M using 126 liver extracts and 43 leukocytes in Japanese.

MATERIALS AND METHODS

Samples

126 liver samples were obtained from the Department of Pathology of Tokyo Women's Medical College. 43 leukocytes were collected from the healthy donors using EDTA-Na tubes. To each liver sample, an equal volume of distilled water was added and thoroughly homogenized. Then it was centrifuged at $15{,}000 \times g$ for 30 min at 4°C. The supernatant was removed for electrophoresis.

Leukocytes were prepared from 10 ml samples of whole blood by washing the buffy coat three times in 10 ml of distilled water. Then the packed leukocytes were lysed by an equal volume of Triton X-100 (10%) solution and mixed softly at 4°C for electrophoresis.

Electrophoresis

The phenotypes of ME_M were detected by starch gel electrophoresis, essentially the same method as described by Siebert *et al.* Electrophoresis was carried out for 20 h using a Tris-histidine X HCl buffer system containing 0.2 M Tris and 0.2 M histidine X HCl (pH 7.6). The gel was a 1 : 7 dilution of bridge buffer (18% hydrolysed starch). Vertical electrophoresis was performed at 5 V/cm with cooling at 4°C.

Staining method

The enzyme specific staining procedures were carried out according to the methods by Hopkinson *et al.* (1976).

Reaction mixtures

i) L-Malic acid (15 mM final conc. in reaction mixture): 100 mg dissolved in 20 ml 0.1 M Tris-HCl buffer, pH 7.0. Then pH readjusted to 7.0 with NaOH.
ii) 0.2 M $MgCl_2$: 2.5 ml
iii) NADP (Na_2 salt) : 5 mg in 1 ml H_2O
iv) MTT : 5 mg in 1 ml H_2O
v) PMS : 0.5 mg in 0.1 ml H_2O
vi) Agar (approx. 2%) : 25 ml

These were mixed rapidly and quickly poured over the sliced starch gel. The gel was incubated at 37°C for 1–2 h. Then the appearance of the dark blue colour is observed and the phenotypes were decided.

RESULTS AND DISCUSSION

Figure 1 shows the malic enzyme phenotypic patterns ME_M^1, ME_M^{2-1}, ME_M^2 obtained from different individuals by starch gel electrophoresis in Tris histidine buffer. Among three phenotypic patterns, the enzyme band of ME_M^2 appeared to be slightly weak stained than that of ME_M^1. The ME_M^{2-1} band consists of the cathodal band of ME_M^2 and the anodal band of ME_M^1. Povey *et al.* (1975) suggested that ME_M isozyme was tetramer and their data using man-mouse and man-hamster cell hybrids would be consistent with ME_M locus being on the 1q chromosome of humans.

Figure 2 is a schematic diagram of ME_M. This electrophoretic patterns were confirmed as ME_M using mitochondria by differential centrifugation of homogenized human liver with 0.25 M sucrose solution. No variations of the soluble form malic enzyme (ME_s) have been reported so far. Table 1 gives the results of the examinations of 126 liver samples and 43 leukocytes from Japanese. The gene frequencies of ME_M^1 and ME_M^2 were estimated as 0.56 and 0.44, respectively. On the basis of Hardy-Weinberg equilibrium, there is an agreement between the observed and the expected frequencies ($\chi^2=0.0023$, d.f.$=1$, $p>0.9$). In our

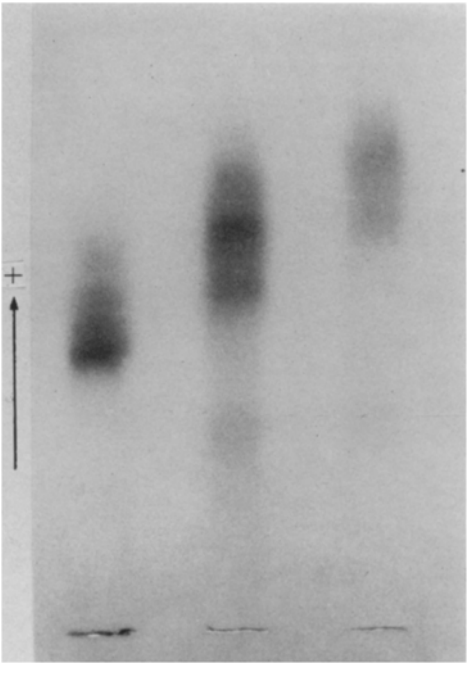

Fig. 1. Photograph of starch gel electrophoretic patterns of mitochondrial malic enzyme.

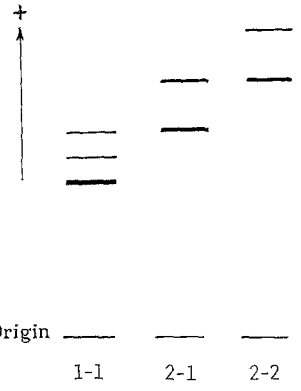

Fig. 2. Schematic diagram of ME_M phenotypes using starch gel electrophoresis.

Table 1. Distribution of ME_M phenotypes and gene frequencies in Japanese.

Total		Phenotypes 1-1	2-1	2-2	
Liver extract 126 samples	Obs. No.	39	62	25	$ME_M^1=0.556$
	%	31.0	49.2	19.8	$ME_M^2=0.444$
	Exp. No.	39.0	62.2	24.8	
	$\chi^2=0.0023$	d.f.=1	$p>0.9$		

Total		Phenotypes 1-1	2-1	2-2
Leukocytes 43 samples	Obs. No.	13	24	8

Table 2. Distribution of ME_M phenotypes between Japanese population and the population of other races.

Author	n	1-1	1-2	2-2	ME_M^1
Cohen and Omenn (North American, 1972)	132	58	67	7	0.67
Cohen and Omenn (American Negroes, 1972)	20	14	5	1	0.83
Povey *et al.* (European Caucasians, 1975)	60	26	30	4	0.69
Burchell *et al.* (European Caucasians, 1977)	66	28	27	11	0.63
Saha *et al.* (European Caucasians, 1978)	409	164	195	45	0.65
Siebert *et al.* (Sourthwestern Germany, 1979)	184	82	82	20	0.67
Ghosh *et al.* (Eastern Indian, 1980)	182	51	102	29	0.56
Abe and Akiyama (Japanese, 1982)	126	39	62	25	0.56

samples, there were no variant types deviating from the three common phenotypes.

Table 2 shows that the frequency of $ME_M^1 = 0.56$ in the Japanese population is much lower than that in American Negroes (0.82), North Americans (0.69), Southwestern Germans (0.67) and European Caucasians (0.65). But this results coincided with the gene frequencies of the Indian population (0.56).

From the results, it was found that the mitochondrial malic enzyme could be applicable in the field of forensic medicine.

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