RED CELL GLYOXALASE I AND PLACENTAL SOLUBLE ACONITASE POLYMORPHISMS IN THE THREE MAJOR ETHNIC GROUPS OF MALAYSIA

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Summary   We surveyed three major ethnic groups in Malaysia for red cell glyoxalase I polymorphism.   The allelic frequencies for GLO^1 were: Malays, 0.196; Chinese, 0.200; and Indians, 0.287.   No rare variant was seen.   By means of gel electrophoresis of placenta samples, the $ACON^2_s$ allele was found to be polymorphic in Chinese and Malays.   The gene frequencies were 0.045 for Chinese and 0.039 for Malays.   Only the common $ACON_s$ 1-1 phenotype was present among the 170 Indians we examined.

INTRODUCTION

Glyoxalase I [EC 4.4.1.5], also known as lactoyl-glutathione-lyase, catalyzes the irreversible conversion of glutathione and methyl-glyoxal to S-lactoyl-glutathione which can then be converted to DL-lactate by glyoxalase II [EC 3.1.2.6], also known as hydroxyacylglutathione hydrolase (Knox, 1960).   Human erythrocytes contain much glyoxalase I and negligible amounts of glyoxalase II (Cohen and Sober, 1945; Valentine and Tanaka, 1961).

Kömpf and Bissbort (1975) described a method for detecting red cell glyoxalase I (GLO) on starch gel and found human GLO to be determined by an autosomal locus with two common alleles $(GLO^1$ and $GLO^2)$.   This locus was later found to be linked to the HLA region on chromosome 6 (Lewis *et al.*, 1976; Weitkamp and Guttormsen, 1976; Meera Khan *et al.*, 1976).

Aconitase [EC 4.2.1.3], one of the enzymes in Krebs's tricarboxylic acid cycle, catalyzes the reversible conversions of citric acid to *cis*-aconitic acid and to isocitric acid.   Of these three tricarboxylic acids, citric acid predominates in cells at pH 7.4 and 25°C.   Dickman and Speyer (1954) reported the presence of both cytoplasmic and mitochondrial aconitases and Koen and Goodman (1969) could distinguish two

forms of aconitase electrophoretically. Aconitase is widely distributed in various animal tissues but neither form is found in red blood cells.

Electrophoretic variation of cytoplasmic aconitase was reported by Koen and Goodman (1969) in slow lorises. In a survey of human placentae from 400 Europeans, Schmitt and Ritter (1974) discovered two variant phenotypes of cytoplasmic aconitase that they attributed to variant alleles present in heterozygous individuals. No mitochrondrial aconitase variant was seen. Slaughter and coworkers (1975), in a survey of placental specimens of both Europeans and Nigerians, found two alleles at $ACON_m$ and seven alleles at $ACON_s$. The variations due to the three most common $ACON_s$ alleles in the Nigerians constituted a polymorphism. Family studies carried out by Slaughter and coworkers showed that these variations were genetically determined.

We report the allelic frequencies of *GLO* and $ACON_s$ in the three major ethnic groups of Malaysia: Malays, Chinese and Indians.

MATERIALS AND METHODS

Blood samples from 592 adult blood bank donors (294 Malays, 183 Indians and 115 Chinese) were obtained from the National Blood Transfusion Service in Kuala Lumpur. The standard method, using distilled water and toluene, was employed to prepare hemolysates.

Starch gel electrophoresis for GLO was based primarily on the method of Harada and Misawa (1976) with two minor modifications: (1) vertical instead of horizontal electrophoresis was employed, and (2) low voltage (6 V/cm) and a longer running time (19 hr) were used. Harada and Misawa's two-step staining method in which the isozymes appeared as colorless bands against a purplish background was used.

Placental tissues, obtained from the Maternity Unit, General Hospital, Kuala Lumpur, were washed and then homogenized with an equal volume of water by means of a glass grinder. The debris was spun down and the supernatant frozen and thawed at least once before use in the electrophoretic analysis of aconitase. Electrophoresis for ACON was performed using the Tris-citrate buffer of Chen and Giblett (1971). The staining was according to Slaughter *et al.* (1975).

RESULTS AND DISCUSSION

The results obtained for glyoxalase I are shown in Table 1. No rare variant was seen. The allelic frequencies for the three groups were: Malays, $GLO^1 = 0.196 \pm 0.016$, $GLO^2 = 0.804 \pm 0.016$; Chinese, $GLO^1 = 0.200 \pm 0.027$, $GLO^2 = 0.800 \pm 0.027$; Indians, $GLO^1 = 0.287 \pm 0.024$, $GLO^2 = 0.713 \pm 0.024$. These allelic frequencies were used to calculate the numbers expected for each phenotype, assuming Hardy-Weinberg equilibrium in each of the three ethnic groups. As shown in Table 1, no significant

Table 1.   Erythrocyte glyoxalase I phenotypes of samples from the
three major ethnic groups of Malaysia.

Ethnic groups	Phenotypes			Total	χ^2	P
	1-1	2-1	2-2			
Malays						
Observed	13	89	192	294	0. 42	>0. 50
Expected	11. 25	92. 52	190. 25			
Chinese						
Observed	7	32	76	115	1. 96	>0. 10
Expected	4. 60	36. 80	73. 60			
Indians						
Observed	14	77	92	183	0. 15	>0. 50
Expected	15. 06	74. 88	93. 06			

deviation from the expected phenotypic numbers was observed in any of the three ethnic groups.

Genetic polymorphism of red cell GLO I was reported in Germans (Kömpf and Bissbort, 1975), in English (Bagster and Parr, 1975) and in Dutch (Meera Khan et al., 1976) with GLO¹ frequencies being 0.43, 0.44 and 0.45, respectively. There is no significant difference in GLO gene frequencies among these Europeans. Olaisen et al. (1976) however, reported that the GLO¹ frequency in Lapps (0.304) was significantly different from that in Norwegians (0.442). Weitkamp and Guttormsen (1976) found the GLO¹ frequency of 0.28 in American Negroes to be much lower than that of American Caucasians (0.42). We found the frequency of GLO¹ in our Indian samples to be similar to those of American Negroes and Lapps.

Ghosh (1977) surveyed over 7,000 persons from populations in South and Southeast Asia, Oceania, Iran and Colombia. The GLO¹ frequencies were very low in most parts of Oceania, including Australia. Some of the populations have no GLO¹ allele while others have it ranging from 0.3% to 15%. Japanese (Harada and Misawa, 1976) also have a low GLO¹ frequency of 8.8%. Ghosh found populations in Southeast Asia to have higher GLO¹ frequencies, with Toba Bataks 15%, Indonesians in the Nias Islands 22%, and Chinese in Singapore 15%. Our data for Malays and Chinese in Malaysia are within this range.

The physiological function of glyoxalase I and II is not known. Szent-Györgyi et al. (1967) speculated that methylglyoxal, the substrate, acts as a physiological inhibitor of cell division and that the enzyme, by destroying this inhibitor, allows cell division to occur. It has not yet been established whether there is increased GLO I or GLO II activity in certain neoplastic tissues. The GLO I and properdin factor B(Bf) loci are linked to the HLA region and both are polymorphic markers. This makes them useful for research in the association of diseases with genetic markers in humans.

A total of 490 placental specimens from 155 Malays, 165 Chinese and 170 Indians were phenotyped for soluble aconitase. Only two alleles were found in Malaysians,

Table 2.   Placental soluble aconitase phenotypes of samples from
the three major ethnic groups of Malaysia.

Ethnic groups	Phenotypes			Total	χ^2	P
	1-1	2-1	2-2			
Malays						
Observed	143	12	0	155	0.25	>0.50
Expected	143.23	11.54	0.23			
Chinese						
Observed	151	13	1	165	1.40	>0.10
Expected	150.34	14.32	0.34			
Indians						
Observed	170	0	0	170		

$ACON_s^1$ and $ACON_s^2$. The variant pattern of $ACON_s$ 2-1 exhibited two bands with equal staining intensities: one zone corresponding in electrophoretic mobility to the single $ACON_s$ 1 band and the other was electrophoretically more anodal. In $ACON_s$ 2 only the more anodal band was seen. The results are presented in Table 2. The allelic frequencies were: Malays, $ACON_s^1 = 0.961 \pm 0.011$, $ACON_s^2 = 0.039 \pm 0.011$; Chinese, $ACON_s^1 = 0.955 \pm 0.011$, $ACON_s^2 = 0.045 \pm 0.011$; Indians, $ACON_s^1 = 1.000$. No significant deviation from the expected phenotypic numbers, assuming Hardy-Weinberg equilibrium, was observed in Malays and Chinese. The variation in $ACON_s^2$ in Chinese and Malays constituted a polymorphism.

In a small number of Orientals (11), Slaughter et al. (1975) found no electrophoretic variation. However, among 60 Indians/Pakistanis they found two individuals with a variant phenotype: one was $ACON_s$ 7-1 and the other gave an $ACON_s$ 5 pattern. The $ACON_s$ 5 pattern was postulated as being either homozygous for the $ACON_s^5$ allele or a heterozygote whose second allele was silent.

In our survey of 170 Indians, not a single variant was seen in line with the known heterogeneity of Indian populations. Most Malaysian Indians originated from southern India where the genetic makeup is quite different from that of northern Indian populations like Sikhs and Punjabis. A more detailed survey of soluble aconitase in northern Indians might reveal the existence of $ACON_s$ 5 as a common variant or if there was a silent allele present.

The similarity of allelic frequencies of $ACON_s^2$ in Malays and Chinese is not surprising as these two populations are closely related. The Malays are believed to have originated in the Yunnan region of China.

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