Am J Hum Genet 32:690–694, 1980

Genetic Polymorphism of Human Erythrocyte Glyoxalase II

P. G. BOARD[1]

SUMMARY

A new method for the detection of glyoxalase II (hydroxyacylglutathione hydrolase) after starch gel electrophoresis is described. A survey of Asian-Pacific populations indicates that genetic polymorphism of glyoxalase II is extremely rare. Polymorphism was observed only in a Micronesian population from the island of Nauru, in which the frequency of the new variant allele *HAGH*^{2} was found to be .016. The electrophoretic pattern in heterozygotes was a double band, suggesting that the structure of glyoxalase II is monomeric.

INTRODUCTION

The glyoxalase pathway converts methylglyoxal to lactic acid by two enzymatic steps in the presence of reduced glutathione (GSH), an essential cofactor:

$$\text{methylglyoxal} + \text{GSH} \xrightarrow{\text{glyoxalase I}} \text{S-lactoyl-glutathione};$$

$$\text{S-lactoyl-glutathione} + H_2O \xrightarrow{\text{glyoxalase II}} \text{D-lactic acid} + \text{GSH}.$$

Glyoxalase I exhibits a high degree of electrophoretic polymorphism with two common alleles in human populations [1, 2]. In comparison, glyoxalase II has been shown to be monomorphic in a study of 606 American whites and 81 American blacks [3].

We have developed an alternate method for the detection of erythrocyte glyoxalase II after starch gel electrophoresis. This paper describes a survey of several Asian-Pacific populations. In one population, from the island of Nauru, we identified a genetic polymorphism characterized by an electrophoretically fast allelic variant.

Received January 15, 1980.

[1] Department of Human Biology, John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., 2601, Australia.

MATERIALS AND METHODS

Samples were collected and stored as packed cells, which were frozen in liquid nitrogen for up to 3 years. Horizontal electrophoresis was carried out in 12% starch gels (Connaught hydrolyzed starch, Willowdale, Ontario, Canada) between metal cooling plates at 8°C. The bridge buffer system contained 0.661 M Tris and 0.083 M citric acid, pH 8.6, and the gel buffer contained 20 ml of the bridge buffer and 50 mg of EDTA in 5 ml, adjusted to pH 8.6, and diluted with 525 ml of water, as described [3].

The zones of glyoxalase II activity were detected by a two-stage procedure. *Stage 1*: The cut surface of the gel is overlayed with filter paper saturated in a solution prepared by combining 30 mg of GSH (Sigma, St. Louis, Mo.), 60 μl methylglyoxal (40% solution, Koch-Light, Colnbrook, England), and 10 U glyoxalase I (grade III, Sigma). The reaction mixture for stage 1 is preincubated at 37°C for at least 15 min before being applied to the gel to allow the formation of S-lactoyl-glutathione, the substrate of the glyoxalase II reaction. After the preincubated reaction mixture is applied to the gel surface, the gel is incubated for 30 min at 37°C. *Stage 2*: The filter paper from stage 1 is removed, and the gel is overlayed with a developing solution prepared by combining 30 ml of iodine solution (2.5 ml of 1% I_2 in KI diluted to 30 ml with water) with an equal volume of 2% molten agar. The agar sets on the gel, and an intense blue starch-iodine color appears immediately over the gel surface except in areas where glyoxalase II has generated GSH from S-lactoyl-glutathione. Zones of glyoxalase II activity appear as colorless areas in a deep-blue background.

RESULTS

The common phenotype observed in all the populations studied consisted of a single band of activity which migrates anodally at pH 8.6. This phenotype has been termed type 1. In many Nauruans, a second phenotype was observed which consisted of two zones of activity, the first identical to type 1, and the second, with a faster electrophoretic mobility. This second phenotype has been termed type 2-1 and is shown in figure 1. The populations surveyed in this study are given in table 1.

The two phenotypes observed in the Nauru population suggest the presence of an allelic variant at the structural locus for glyoxalase II. We, therefore, propose to refer to this system as the *HAGH* locus with two alleles: $HAGH^1$ for the common slow-anodal form and $HAGH^2$ for the fast-anodal form. This proposed model includes

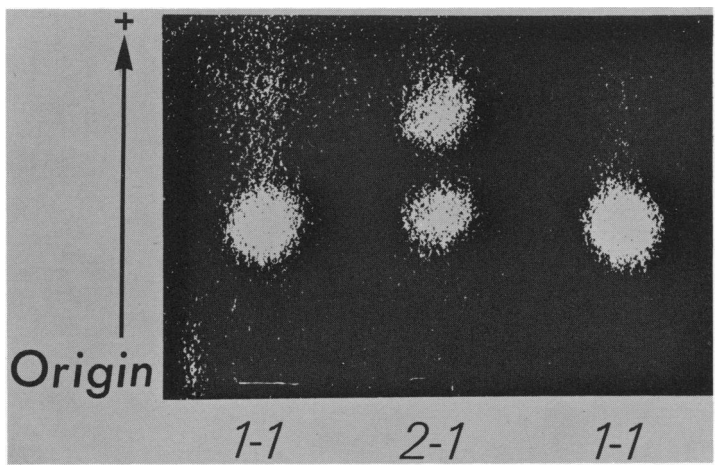

FIG. 1.—Observed phenotypes of human erythrocyte glyoxalase II (*HAGH*)

TABLE 1

POPULATIONS SURVEYED COMPOSED ENTIRELY OF THE 1-1 PHENOTYPE

Population	No.
Caroline Islands:	
Eastern	126
Western	126
Tokelau Islands	132
Western Samoa	96
Loyalty Islands	96
Papua New Guinea	96
Australian Aboriginal	96
Singapore:	
Chinese	45
Indian	10
Malay	13

the possibility of a third phenotype, representing the type 2 homozygote which was not observed. Observed phenotype numbers shown in table 2 do not differ significantly from those expected under the assumption of Hardy-Weinberg equilibrium (χ^2 = 0.14). Family data (fig. 2) indicate that the observed electrophoretic variation of glyoxalase II is genetically transmitted, and the presence of male heterozygotes and father-to-son transmission clearly indicates that the *HAGH* locus is autosomal. The double-banded electrophoretic pattern of the heterozygote (2-1) is characteristic of a monomeric protein.

DISCUSSION

Our initial studies using the method described by Charlesworth [3] for the localization of glyoxalase II after electrophoresis were unsuccessful, probably because oxidized GSH is not a good substrate for glyoxalase I and a specific D-lactate dehydrogenase is required to link the reaction to the reduction of NAD. Consequently, we developed an alternate method which detects the release of GSH from S-lactoyl-glutathione. It is the inverse of a procedure designed to detect the loss of GSH catalyzed by the glyoxalase I reaction [4] and has proved very sensitive and can readily detect glyoxalase II from whole blood samples stored on dried filter paper strips.

This investigation, together with Charlesworth's [3], shows that glyoxalase II polymorphism is extremely rare. Our data show that a genetic polymorphism exists only in the Nauruan population and that the two alleles are inherited in an autosomal

TABLE 2

PHENOTYPES OF GLYOXALASE II IN THE NAURUAN POPULATION

	HAGH 1	HAGH 2-1	HAGH 2
Observed (no. = 526)	509	17	0
Expected	509.14	16.76	0.14

NOTE. —Gene frequency $HAGH^1$ = .9838; $HAGH^2$ = .0162.

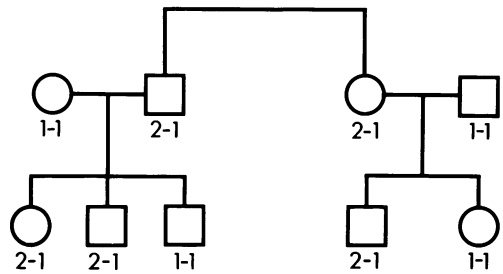

FIG. 2. —Family data showing genetic transmission of *HAGH*^{1} and *HAGH*^{2} alleles

codominant manner. The people of Nauru are classified as Micronesian. Other Micronesians studied (Caroline Islands) failed to show any evidence of this polymorphism. Its restriction to individuals from the island of Nauru suggests that a relatively recent mutation probably gave rise to this variation.

There have been few studies of glyoxalase II from human tissues. Purification of glyoxalase II from human liver has revealed it to be a basic protein (isoelectric point [pI] 8.35) with a mol. wt. of 22,900 [5]. Banding patterns of the heterozygotes in our study suggest that the enzyme consists of a single polypeptide chain.

Erythrocyte glyoxalase II deficiency has been previously reported in one kindred, coincidental with hereditary elliptocytosis [6]. There was no evidence in those cases that glyoxalase II deficiency had any deleterious effects on erythrocyte viability. In the present investigation, the activity of glyoxalase II in fresh samples has not been quantitated. However, the staining intensity of the *HAGH*^{2} product does not appear to be diminished when compared with the common *HAGH*^{1} product. Considering the absence of adverse clinical effects of homozygous erythrocyte glyoxalase II deficiency, this polymorphism probably has no significant physiological consequences.

A recent report [7], received after the completion of this work, also described an alternate method for the localization of glyoxalase II after electrophoresis and isoelectric focusing, and discussed the disadvantages of Charlesworth's method [3]. It also reported multiple forms of glyoxalase II in human erythrocytes, but did not indicate the presence of any genetic polymorphism. In our study, a single-banded electrophoretic pattern was the commonly observed phenotype. Our failure to resolve a multicomponent pattern was probably due to the use of entirely different gel and buffer systems.

ACKNOWLEDGMENTS

I am grateful to Ms. M. Coggan for her excellent technical assistance, to Professor Paul Zimmet, Royal Southern Memorial Hospital, for providing access to the Nauru samples, and to Ms. Sonny Whitehouse for compiling the pedigree information. Dr. R. L. Kirk made many helpful suggestions during the preparation of the manuscript.

REFERENCES

1. KÖRMPF J, BISSBORT S, GUSSMANN S, RITTER H: Polymorphism of red cell glyoxalase I (E.C.4.4.1.5.): a new genetic marker in man. Investigation of 169 mother-child combinations. *Humangenetik* 27:141–143, 1975

2. GHOSH AK: Polymorphism of red cell glyoxalase I with special reference to South and Southeast Asia and Oceania. *Hum Genet* 39:91–95, 1977
3. CHARLESWORTH D: Starch gel electrophoresis of 4 enzymes from human red blood cells; glyceraldehyde-3-phosphate dehydrogenase, fructose-aldolase, glyoxalase II, and sorbitol dehydrogenase. *Ann Hum Genet* 35:477–484, 1972
4. PARR CW, BAGSTER IA, WELCH SG: Human red cell glyoxalase I polymorphism. *Biochem Genet* 15:109–113, 1977
5. UOTILA L: Purification and characterization of S-2-hydroxyacylglutathione hydrolase (glyoxalase II) from human liver. *Biochemistry* 12:3944–3951, 1973
6. VALENTINE WN, PAGLIA DE, NEERHOUT RC, KONRAD PN: Erythrocyte glyoxalase II deficiency with coincidental hereditary elliptocytosis. *Blood* 36:797–808, 1970
7. UOTILA L: Glutathione thiol esterases of human red blood cells. *Biochim Biophys Acta* 580:277–288, 1979