*Amer J Hum Genet* 25:382–387, 1973

# Hemoglobin Constant Spring (Slow-moving Hemoglobin X Components) and Hemoglobin E in Malayan Aborigines

LIE-INJO LUAN ENG,[1] A. BAER, A. N. LEWIS, AND Q. B. WELCH

An abnormal slow-moving hemoglobin consisting of two components designated as slow-moving hemoglobin (Hb) X components [1, 2] was found in a group of patients with Hb H disease in Malaysia. Family studies of many of these patients revealed that invariably one parent had the same abnormal components. It was thought that the combination of this abnormality with $\alpha$ thalassemia leads to Hb H disease [1, 2].

Slowly migrating hemoglobin components were earlier reported in patients with Hb H disease in Greece (Hb Athens [3]) and in Thailand (Hb Thai [4]). However, the significance and mode of inheritance of these components were not elucidated as they were in the report from Malaysia.

Milner, Clegg, and Weatherall [5] and Clegg, Weatherall, and Milner [6] carried out structural studies on similar small, slow-moving components in members of a Chinese family with Hb H disease from Constant Spring, Jamaica. They found that the slow-moving components had abnormal $\alpha$ chains with 172 residues instead of the usual 141; the additional 31 amino acids were attached to the C-terminal end of an otherwise normal $\alpha$ chain. The hemoglobin was designated Constant Spring (Hb CoSp).* Structural comparison of our abnormal slow-moving Hb X components found in Chinese and Malays with Hb CoSp showed them to be identical [7, 8] and also identical to the slow-moving components found in Hb H disease in Greece, Thailand, and Hong Kong [7].

A study of different racial groups in the general population in Malaysia further revealed that this abnormality occurs in more than 2% of Malays, less than 1% of Chinese, but rarely in Indians [9]. Apparently, this abnormality was overlooked in previous surveys of abnormal hemoglobins because of less sensitive methods in use at the time.

This paper reports the results of a survey to estimate the frequencies of Hb CoSp

Received November 29, 1972; revised January 25, 1973.

This work was supported by research grant HL 10486 and University of California International Center for Medical Research and Training (UC ICMRT) research grant AI 10051 from the U.S. Public Health Service, National Institutes of Health.

[1] All authors: Institute for Medical Research and the University of California International Center for Medical Research and Training (UC ICMRT), Kuala Lumpur, Malaysia.

* Earlier papers have used the abbreviation Hb CS. To avoid confusion with sickle cell Hb C disease (also abbreviated Hb CS), the symbol Hb CoSp will be used.

and other abnormal hemoglobins in Malayan aborigines, particularly the Temuan. Although many reports on the frequencies of abnormal hemoglobins in Malayan aborigines have been published [10–13], most concern the Temiar, Semai, and Semelai aboriginal groups and did not look for the presence of the slow-moving Hb X components. One study [10] noted that the frequency of Hb E in a small number of Temuan was much lower than in the Temiar, Semai, and Semelai groups. Also, an unpublished survey of 55 Temuan from the Kuala Langat area in Selangor, West Malaysia (carried out by Lie-Injo in collaboration with the Malaria Division, Institute for Medical Research, Kuala Lumpur), showed that none had abnormal Hb E.

## MATERIALS AND METHODS

Blood samples from 406 Temuan villagers from four different areas in the states of Selangor and Negri Sembilan in West Malaysia and 524 patients of various aboriginal groups from Ulu Gombak Hospital were examined. Of the villagers, 140 were school children ranging in age from about 5 to 12 years. The remaining 266 villagers, of all ages, were sampled at random. No attempt was made to exclude relatives because of the small total number of village inhabitants (40–250). The group of hospital patients consisted of 95 Temuan, 116 Jakun, 233 Semai, and 80 Temiar.

Blood obtained by finger prick was collected in heparinized capillary tubes; venous blood was obtained from 317 of the hospital patients and collected in acid citrate dextrose solution. The samples were immediately refrigerated and hemolysates were prepared within 24 hr. To prepare hemolysates from the capillary blood, the method of Scott [14] was used with slight modifications. Concentrated hemolysates were prepared from the venous blood samples by the method described earlier [9]. Abnormal hemoglobin was studied by starch gel electrophoresis using tris-EDTA-boric acid buffer at *p*H 8.6 and discontinuous tris-boric acid buffer at *p*H 9.5 [15]; each sample was run in both buffers. Hemoglobin patterns were stained for a prolonged period with benzidine and hydrogen peroxide.

## RESULTS AND DISCUSSION

Our findings (tables 1 and 2) indicate that Hb CoSp (slow-moving Hb X components) is more common in the Temuan and Jakun groups of Malayan aborigines than in any other racial groups in Malaysia so far examined. The frequencies in these two groups were even higher than the 2.2% frequency found in Malays [9]. We did not find Hb CoSp in the more than 200 Semai and 80 Temiar aborigines examined. The frequencies of Hb CoSp reported in this study were obtained from

TABLE 1

ABNORMAL HEMOGLOBIN IN MALAYAN ABORIGINE PATIENTS

| Ethnic Group | *N* | Hb A/Hb A | Hb A/Hb E | Hb E/Hb E | Hb A/Hb CoSp |
|---|---|---|---|---|---|
| Temuan | 95 | 90 | 1 | 0 | 4 (4.2%) |
| Jakun | 116 | 109 | 4 | 0 | 3 (2.6%) |
| Semai | 233 | 151 | 68 | 14 | 0 |
| Temiar | 80 | 37 | 35 | 8 | 0 |
| Total | 524 | 387 | 108 | 22 | 7 |

TABLE 2

ABNORMAL HEMOGLOBIN IN MALAYAN TEMUAN ABORIGINES

| | *N* | Hb A/Hb A | Hb A/Hb E* | Hb E/Hb E | Hb A/Hb CoSp† |
|---|---|---|---|---|---|
| Patients‡ ..... | 95 | 90 | 1 | 0 | 4 |
| Villagers ..... | 406 | 382 | 12 | 0 | 12 |
| Patients + villagers .... | 501 | 472 | 13 (2.6%) | 0 | 16 (3.2%) |

* Gene frequency of Hb E = .013.
† Gene frequency of Hb CoSp = .016.
‡ Same Temuan patients as listed in table 1.

relatively dilute hemolysates since highly concentrated hemolysates could not be prepared from capillary blood. As pointed out previously [2, 9], Hb CoSp is easily overlooked in more dilute hemolysates, although prolonged staining increases the sensitivity of this method. Thus the frequencies obtained in this series may be slightly underestimated. However, when these results were compared with those obtained for the concentrated hemolysates prepared from venous blood, no increase in frequency was noted.

As mentioned earlier, the slow-moving hemoglobin components can be detected in trait carriers only with strict attention to the method and chemical composition of the buffer; slight changes in the physical and chemical environment in which the components are studied may have a great influence upon their detectability [9].

The positive samples obtained from capillary blood showed the abnormal components very clearly (fig. 1). We have the impression that the slow-moving hemoglobin components are usually quite pronounced in Malays and Malayan aborigines while in Chinese they are usually, although not always, less easily demonstrable. For this reason, Lie-Injo, Lopez, and Lopes [2] designated them Hb X components rather than assigning them a specific name. However, structural studies have shown that the slow-moving components in Malays are the same as those in Chinese and are identical with Hb CoSp.

In Hb H disease associated with the X components, the relative amounts of Hb $X_1$ and $X_2$ (identical with Hb $CoSp_1$ and $CoSp_2$, respectively), Hb $A_2$, and also a component with slightly more anodic mobility than Hb $A_2$ sometimes seen in Hb H disease depend not only on the freshness of the hemolysate but also upon the method and chemical composition of the buffers used for study [2]. This phenomenon was also observed in two cases of individuals homozygous for the gene associated with the slow-moving components ([8]; L. E. Lie-Injo, unpublished observation). It was suggested that Hb $CoSp_2$ is produced by proteolysis of Hb $CoSp_1$ [6]. Likewise, other electrophoretic components may be produced in the hemolysate. Indeed, in the homozygous condition four to five small components could be demonstrated.

In one village in which six of 166 persons examined had Hb CoSp, all six belonged to the same family. The mother and five of her seven children were Hb

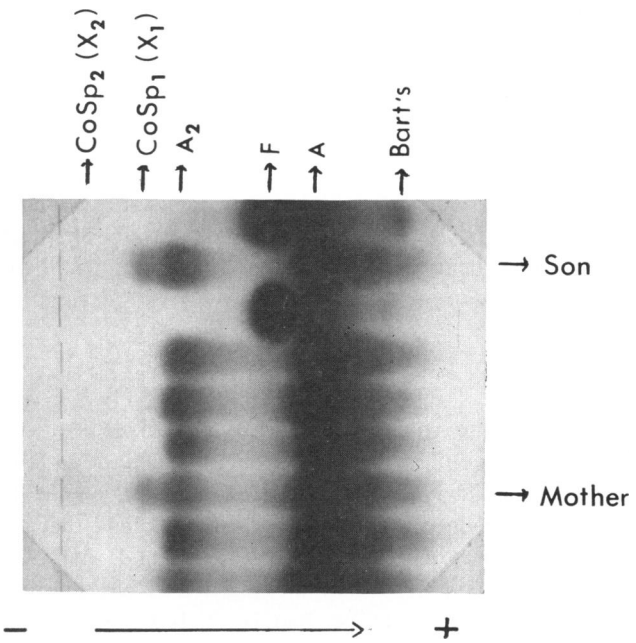


FIG. 1.—Starch gel electrophoresis in tris-EDTA-boric acid buffer, *p*H 8.6, showing the hemoglobin patterns of a Temuan and his mother with Hb CoSp and controls (benzidine stain).

CoSp trait carriers; the father was not. Family studies of two other unrelated trait carriers of Hb CoSp also showed the inheritance pattern to be that of an autosomal codominant gene, as demonstrated earlier in other racial groups [2, 8, 9].

That Hb CoSp, reported in Malaysia as the slow-moving Hb X components, can lead to Hb H disease in Malayan aborigines when combined with thalassemia was reported earlier [2].

According to Williams-Hunt [16], the Malayan aborigines are comprised of three main groups, the Negritos, Senoi, and aboriginal Malays, each consisting of many subgroups. Of the Senoi, the Semai and Temiar subgroups are the most important. The aboriginal Malays, sometimes referred to as Jakun, consist of the Temuan, Jakun proper, Semelai, and many other smaller groups. Although it was earlier suggested that the language of the Temuan in certain areas was basically Senoi [17, 18], the data on abnormal hemoglobins substantiate the greater affinity of the Temuan and Jakun to Malays because of their relatively low Hb E frequency and appreciable frequency of Hb CoSp; the Senoi group has extremely high Hb E frequency and either absent, or if present, very low frequency of Hb CoSp.

Fix [19] showed that the overall frequency of Hb E in the Semai does not agree with the frequencies computed separately for the various villagers in his study. The villages differed considerably in this respect, indicating genetic microdifferentiation resulting from the relative genetic isolation of groups in different villages. Baer et al. will discuss the same phenomenon in the Temuan population of our study (in preparation).

Although no data are presently available on frequencies of Hb CoSp in the general population in Thailand and Hong Kong, they are expected to be appreciable since Wasi et al. [20] found 44% of patients with Hb H disease to have Hb CoSp in Thailand and Todd found 12% of such patients in Hong Kong [21]. These percentages are lower than those found in Malaysia, where more than half of Hb H disease patients had the slow-moving components [1, 2]. Efremov et al. [22] studied small, slow-moving hemoglobin components in members of a Chinese family with Hb H disease in Augusta, Georgia, and partially characterized the abnormal $\alpha$ chain (probably identical to Hb CoSp); they did not find the abnormality in several thousand normal individuals in the area but did find it in one other Chinese family.

SUMMARY

A survey to estimate the frequencies of the abnormal hemoglobin Constant Spring (Hb CoSp; slow-moving Hb X components) and other abnormal hemoglobins was carried out in Malayan aborigines with emphasis on the Temuan group. Among 501 Temuan, the frequency of Hb CoSp was 3.2% and that of Hb E, 2.6%. In 116 Jakun, the frequency of Hb CoSp was 2.6% and that of Hb E, 3.4%. Among the 233 Semai studied, Hb CoSp was not found but the Hb E frequency was 35.2%. In the Temiar group 43.8% had Hb E but none had Hb CoSp. The frequencies of abnormal hemoglobins suggest that the Temuan are more closely related to the Malays than to the Senoi group.

ACKNOWLEDGMENTS

We thank the Jabatan Orang Asli (Office for Aboriginal Affairs) for permission to carry out this survey; Dr. J. M. Bolton and other colleagues of the Hospital Orang Asli Gombak for encouragement and help; the Malaria Division, Institute for Medical Research, Kuala Lumpur, for its kind cooperation; all laboratory and field assistants involved in this work for their valuable help; and especially all aborigines who donated blood.

REFERENCES

1. LIE-INJO LE: Annual report of the University of California International Center for Medical Research and Training, San Francisco, 1970, pp 17–24
2. LIE-INJO LE, LOPEZ CG, LOPES M: Inheritance of haemoglobin H disease: a new aspect. *Acta Haemat* (Basel) 46:106–120, 1971
3. SOFRANIADOU K, KALTSOYA A, LOUKOPOULOS D, FESSAS P: Hemoglobin "Athens": an alpha-chain variant with unusual properties, in 12th Congress International Society of Haematology, New York, 1968, p 56
4. WASI P, NA-NAKORN S, POOTRAKUL S, SOOKANEK M, DISTHASONGCHAN P, PORNPATKUL M, PANICH V: Alpha- and beta-thalassemia in Thailand. *Ann NY Acad Sci* 165:60–82, 1969
5. MILNER PF, CLEGG JB, WEATHERALL DJ: Haemoglobin H disease due to a unique haemoglobin variant with an elongated $\alpha$ chain. *Lancet* 1:729–732, 1971
6. CLEGG JB, WEATHERALL DJ, MILNER PF: Haemoglobin Constant Spring—a chain termination mutant? *Nature* (London) 234:337–340, 1971
7. FESSAS PH, LIE-INJO LE, NA-NAKORN S, TODD D, CLEGG JB, WEATHERALL DJ: Identification of slow-moving haemoglobins in haemoglobin H disease from different racial groups. *Lancet* 1:1308–1310, 1972

8. LIE-INJO LE: Haemoglobin Bart's and slow-moving haemoglobin X components in newborns: the homozygous state for the slow-moving X components in a Malay boy. *Acta Haemat* (Basel) 49:25–35, 1973
9. LIE-INJO LE, DURAISAMY G: Slow-moving haemoglobin X components in Malaysians. *Hum Hered* 22:118–123, 1972
10. LIE-INJO LE, CHIN J: Abnormal haemoglobin and glucose-6-phosphate dehydrogenase deficiency in Malayan aborigines. *Nature* (London) 204:291–292, 1964
11. LIE-INJO LE: Hereditary ovalocytosis and haemoglobin E-ovalocytosis in Malayan aborigines. *Nature* (London) 208:1329, 1965
12. LIE-INJO LE, BOLTON JM, FUDENBERG HH: Haptoglobins, transferrins and serum gamma globulin types in Malayan aborigines. *Nature* (London) 215:777, 1967
13. LIE-INJO LE, FIX A, BOLTON JM, GILMAN RH: Haemoglobin E hereditary elliptocytosis in Malayan aborigines. *Acta Haemat* (Basel) 47:210–216, 1972
14. SCOTT RB: Rapid technic for preparation of hemoglobin solutions for electrophoresis. *Amer J Clin Path* 54:78–81, 1970
15. HAUT A, TUDHOPE GR, CARTWRIGHT GE, WINTROBE MM: The nonhemoglobin erythrocytic proteins, studied by electrophoresis on starch gel. *J Clin Invest* 41:579–587, 1962
16. WILLIAMS-HUNT PDR: *An Introduction to the Malayan Aborigines.* Kuala Lumpur, Government Press, 1952
17. WILKINSON RJ: *Papers on Malay Subjects*, suppl. *The Aboriginal Tribes,* edited by WALLACE JE, Kuala Lumpur, Government Press, 1926
18. LEBAR FM, HICKEY GC, MUSGRAVE JK: *Ethnic Groups of Mainland Southeast Asia.* New Haven, Conn., Human Relations Area Files Press, 1964
19. FIX A: Semai Senoi population structure and genetic microdifferentiation. Doctoral thesis, University of Michigan, 1971
20. WASI P, NA-NAKORN S, POOTRAKUL P, PANICH V: Incidence of haemoglobin Thai: a re-examination of the genetics of $\alpha$ thalassaemia diseases. *Ann Hum Genet* 35:467–470, 1972
21. TODD D: Slow-moving haemoglobin bands in haemoglobin H disease. *Lancet* 2:439, 1971
22. EFREMOV GD, WRIGHTSTONE RN, HUISMAN THJ, SCHROEDER WA, HYMAN C, ORTEGA J, WILLIAMS K: An unusual hemoglobin anomaly and its relation to $\alpha$ thalassaemia and hemoglobin-H disease. *J Clin Invest* 50:1628–1636, 1971

## Research Grants—The National Foundation

The National Foundation–March of Dimes will entertain a limited number of research proposals for the support of basic and clinical research in birth defects. The National Foundation defines a birth defect as an abnormality of structure, function, or metabolism, whether genetically determined or a result of environmental interference during embryonic or fetal life. Requests dealing with the structure and function of chromosomes, their subunits, genes, supporting structures, repressor substances, and the like, will be encouraged. For further information contact Director of Research, The National Foundation–March of Dimes, 1275 Mamaroneck Avenue, White Plains, New York 10605.